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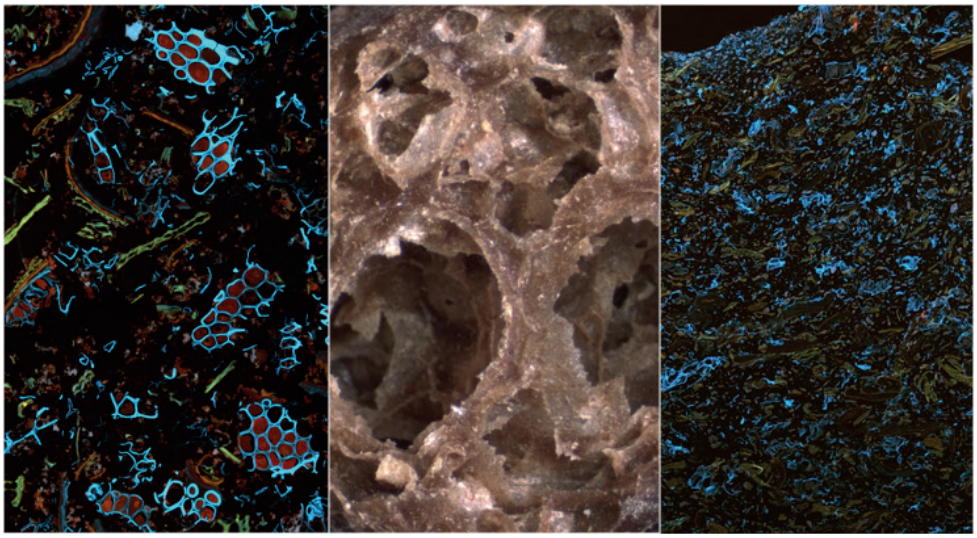
SYED ARIFUL ALAM

PROCESS-INDUCED STRUCTURAL PROPERTIES AND STARCH DIGESTIBILITY OF HIGH-FIBRE EXTRUDED PRODUCTS



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Process-induced structural properties and starch digestibility of high-fibre extruded products

Syed Ariful Alam

DOCTORAL DISSERTATION

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Custos: Associate Professor Kati Katina
Department of Food and Nutrition
University of Helsinki, Finland

Supervisors: Research Professor Nesli Sozer
VTT Technical Research Centre of Finland Ltd

Senior Advisor Kaisa Poutanen
VTT Technical Research Centre of Finland Ltd

Associate Professor Kati Katina
Department of Food and Nutrition
University of Helsinki, Finland

Pre-examiners: Professor Maud Langton
Department of Molecular Sciences
Swedish University of Agricultural Sciences, Sweden

Dr. Pekka Lehtinen
CEO, Founder
Lesetoils Oy, Finland

Opponent: Professor Charles Brennan
Food Science and Nutrition
Lincoln University, New Zealand

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Abstract

This study focused on modification of rye bran to produce high fibre extruded cereal foods with a good texture and structure. Rye bran addition during extrusion is challenging due to high levels of insoluble dietary fibre, which leads to less expanded products and a hard texture. Bran modification by particle size reduction or fermentation significantly improved both the structural and textural properties of extrudates. Moreover, optimization of the processing parameters such as increasing the screw speed, lowering the water feed rate, as well as the use of in-barrel hydration regimens further improved the textural properties. The applicability of rye bran in extruded products could thus be improved by particle size reduction and fermentation.

The extruded food structure and texture had a direct effect on the mastication and bolus formation process in the mouth. A hard and dense extrudate structure required more mastication effort than a crispy structure. Crispy and porous structures easily disintegrated in the mouth and produced smaller bolus particles than a hard and dense structure. A smaller particle size of the bolus was associated with increased starch hydrolysis. The bolus particle size was more effective than the matrix composition in altering the starch digestibility.

Increased dietary fibre intake via appealing snack products could help reduce chronic diseases. Knowledge obtained in this thesis on cereal matrix formation and digestion and the effects of added dietary fibre on the structural and textural properties of extruded solid foams will help the food industry to develop healthy and appealing products. Understanding process-structure-digestibility relationships of high fibre extruded matrices is essential for designing health promoting foods.

Keywords Cereal foods, crispiness, dietary fibre, electromyography, expansion, extrusion, fermentation, flakes, microstructure, X-ray microtomography, particle size reduction, puffs, rye bran, rye-based extrudates, mastication, starch digestion, structure, texture

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Tiivistelmä

Tässä tutkimuksessa keskityttiin ruisleseen muokkaamiseen niin, että siitä voitaisiin valmistaa runsaskuituisia ekstrudoituja viljatuotteita, joilla on hyvä rakenne. Ruiskuidun lisääminen ekstruusio-prosessoinnissa on haasteellista, sillä se sisältää runsaasti liukenematonta ravintokuitua, mikä johtaa vähemmän huokoisiin tuotteisiin ja kovaan rakenteeseen. Leseen muokkaaminen sen partikkelikokoa pienentämällä tai maitohappokäymisen avulla paransi huomattavasti lopputuotteiden rakennetta. Lisäksi prosessiolosuhteiden optimointi, esimerkiksi ekstruuderin ruuvin kiertonopeuden kasvattaminen, veden syöttönopeuden alentaminen ja ekstruusio prosessin vesipitoisuuden säätely edelleen paransivat tuotteiden ominaisuuksia. Ruisleseen esikäsittely partikkelikokoa pienentämällä ja tuottamalla siihen dekstraania Weissella confusa maitohappokäymisen avulla paransivat siis huomattavasti leseen soveltuvuutta ekstruusiotuotteiden valmistukseen.

Ekstrudoitujen tuotteiden rakenne vaikutti pureskeltavuuteen ja pureskellun ruokamassan muodostumiseen suussa. Kova ja tiivis rakenne vaati enemmän pureskeluvoimaa kuin rapea rakenne. Rapea ja huokoinen rakenne hajosi helposti suussa pienemmiksi partikkeleiksi, mikä liittyi tärkkelyksen lisääntyneeseen hydrolyysiin. Ruokamassan partikkelikoko pureskelun jälkeen oli tärkeämpi ruuan sulamisnopeudelle kuin leseen koostumus.

Ravintokuidun saannin lisääminen kuluttajia miellyttävien välipalojen avulla voisi auttaa vähentämään kroonisia sairauksia. Väitöskirjassa syntynyt tieto viljatuotteiden rakenteen muodostuksesta ja sulamisesta, samoin kuin lisätyn ravintokuidun vaikutuksista ekstrudoitujen kiinteiden vaahtomaisten tuotteiden rakenteeseen, auttaa elintarviketeollisuutta kehittämään terveellisiä ja kuluttajia houkuttelevia tuotteita. Runsaskuituisten ekstrudoitujen tuotteiden prosessoinnin, rakenteen ja sulavuuden välisten suhteiden ymmärtäminen on keskeistä kehitettäessä terveyttä edistäviä elintarvikkeita.

Keywords Cereal foods, crispiness, dietary fibre, electromyography, expansion, extrusion, fermentation, flakes, microstructure, X-ray microtomography, particle size reduction, puffs, rye bran, rye-based extrudates, mastication, starch digestion, structure, texture

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Helsinki, November 2020

Syed Ariful Alam

List of original publications

This thesis is based on the following publications:

I Alam, S. A., Järvinen, J., Kirjoranta, S., Jouppila, K., Poutanen, K., & Sozer, N. (2014). Influence of particle size reduction on structural and mechanical properties of extruded rye bran. *Food and Bioprocess Technology 7*(7): 2121−2133.

II Alam, S. A., Järvinen, J., Kokkonen, H., Jurvelin. J., Poutanen, K., & Sozer, N. (2016). Factors affecting structural properties and *in vitro* starch digestibility of extruded starchy foams containing bran. *Journal of Cereal Science 71*: 190−197.

III Alam, S. A., Pentikäinen, S., Närväinen, J., Holopainen-Mantila, U., Poutanen, K., & Sozer, N. (2017). Effects of structural and textural properties of brittle cereal foams on mechanisms of oral breakdown and *in vitro* starch digestibility. *Food Research International 96*: 1−11.

IV Alam, S. A., Pentikäinen, S., Närväinen, J., Katina, K., Poutanen, K., & Sozer N. (2019). The effect of structure and texture on the breakdown pattern during mastication and impacts on *in vitro* starch digestibility of high fibre rye extrudates. *Food & Function 10*(4): 1958−1973.

The publications are referred to in the text by their numbers instead of roman numerals.

Author's contributions

I The author was responsible for planning the study together with other authors, but the original idea came from Professor Nesli Sozer and Professor Kaisa Poutanen. The author was responsible for the execution of the experimental work and data analysis. The author conducted the extrusion with the help of Dr. Satu Kirjoranta and a trainee Jenni Järvinen. Dietary fibre analyses were performed with the help of technicians. Dr. Harri Kokkonen carried out the X-ray microtomography analysis. Syed Ariful Alam interpreted the results and had the main responsibility for writing the publication together with all co-authors.

II The author planned the work together with the other authors especially with his supervisor Professor Nesli Sozer and Professor Kaisa Poutanen. Syed Ariful Alam was responsible for the execution of the experimental work and data analysis. The author conducted the extrusion together with Jenni Järvinen and with the assistance of Dr. Satu Kirjoranta. Dr. Harri Kokkonen carried out the X-ray microtomography analysis. Syed Ariful Alam interpreted the results and wrote the publication in cooperation with the other authors.

III The author was responsible for planning the work together with Professors Nesli Sozer and Kaisa Poutanen. The author conducted the extrusion with the help of technicians. Syed Ariful Alam and Saara Pentikäinen had the responsibility for running the mastication trials with electromyography (EMG). Dr. Johanna Närväinen was responsible for retrieving the EMG data. Syed Ariful Alam had the responsibility for further calculation and interpretation of the EMG data. Professor Jukka Jurvelin provided Xray microtomography facilities. The author interpreted the results together with Professor Nesli Sozer and wrote the publication in collaboration with all co-authors.

IV The author planned the work together with other authors especially with his supervisor Professor Nesli Sozer and Professor Kaisa Poutanen. A research scientist from VTT assisted with the EPS-fermentation of the rye bran. Syed Ariful Alam was responsible for extrusion and planning the mastication trials (EMG) and sensory analyses with the help of Dr. Saara Pentikäinen. Dr. Johanna Närväinen had the responsibility for retrieving the EMG data. The author interpreted the results and wrote the publication in cooperation with all co-authors.

Abbreviations

AUC	Area under the curve
BMI	Body Mass Index
Ci	Crispiness index
Cw	Crispiness work
D	Average cell diameter
DF	Dietary fibre
EMG	Electromyography
EPS	Exopolysaccharide
FB	Fermented rye bran
f-d	Force-displacement curve
Fmax	Maximum force in the force-displacement curve
GI	Glycaemic index
HI	Hydrolysis index
IB	In barrel-water feed
IDF	Insoluble dietary fibre
LAB	Lactic acid bacteria
PC	Preconditioning
RB	Rye bran
RF	Endosperm rye flour
SDF	Soluble dietary fibre
SME	Specific mechanical energy
t/D	Average cell wall thickness to cell diameter ratio
TDF	Total dietary fibre
TTA	Titratable Acidity
XMT	X-ray microtomograpy

Contents

1 Introduction

Rye (Secale cereale L.) is a widely cultivated cereal grain in Northern and Eastern Europe and is a key source of dietary fibre (DF) in the Nordic countries (Jonsson et al., 2018). It comprises about 40% of dietary fibre sources in Finland (Juntunen et al., 2000). Health benefits such as reduced risk of cardiovascular diseases, cancer, type II diabetes and obesity are associated with the consumption of food products rich in high DF (Livesey et al., 2008; Dahm et al. 2010; Smith & Tucker, 2011). Despite beneficial health effects, the consumption of DF in Western countries remains below the recommended (25–35 g/day) level (Jonsson et al., 2018).

In recent years, snack foods have become a part of the prevailing lifestyle in developed countries. Snack foods (e.g. cellular solid foams) are generally made of refined flours such as corn, wheat and rice thus lacking nutritional quality and DF (Robin et al., 2012b; Brennan et al., 2013). Therefore, the production of snack foods with high DF has gained interest in recent years. According to a recent market research report, the forecasted consumption of extruded snacks alone would be €55.3 billion by the year 2026 (MarketsandMarkets, 2020). Increasing market demand and consumer choice towards healthy snacks has led food engineers to develop novel snack foods rich in DF by replacing conventional raw materials such as potato and corn (Brennan et al., 2013; MarketsandMarkets, 2020; Singh & Vijay-Kumar, 2020).

Extrusion is a short, high-temperature processing technique to produce snack and convenience foods. A variety of ready to eat products, e.g. breakfast cereals, snacks and pasta can be produced using extrusion processing (Lobato et al., 2011; Sozer & Poutanen, 2013). Extrusion processing alters the functional properties of food ingredients and texturizes them. Furthermore, extrusion processing has the potential to improve the nutritional quality of food through starch gelatinisation, the Maillard reaction, enzyme denaturation and through the redistribution of DF (Singh et al., 2007). The physicochemical properties of extruded foods strongly depend on the raw material composition and on the extrusion process parameters. The addition of fibre or bran in extruded food poses both technological and functional challenges by interfering with the continuity of the food matrix. An increased amount of fibre results in extrudates with reduced expansion and crispiness and with increased hardness and density (Desrumaux et al., 1999; Liu et al., 2000).

Mastication is the first stage of digestion, by which food disintegrates into small particles and mixes with saliva to prepare a swallowable bolus. The food structure and matrix components determine the consistency of the bolus as well as the rate of digestion in the gastrointestinal tract (Bornhorst & Singh, 2012). Food undergoes different physical changes during oral processing, for instance, the hardness and particle size of the food decreases, whereas the adhesiveness and cohesiveness increases (Peyron et al., 2011). The mastication time and the number of chews required to prepare a swallowable bolus depend on the moisture content as well as the hardness of the food (Engelen et al., 2005; Hiiemae et al., 1996). The disintegration of the food

structure is an important determinant of digestion in the stomach. Structure disintegration controls gastric emptying and therefore influences the rate of digestion (Bornhorst & Singh, 2012).

2 Review of the literature

2.1 Rye

Rye has the highest amount of DF and bioactive compounds compared to other cereals (Andersson et al., 2009; Koistinen et al., 2018). Therefore, foods made of rye have potential health benefits for reducing the risk of a number of chronic diseases (Jonsson et al., 2018). Rye foods have well-established beneficial effects on the blood glucose response and insulin metabolism and thus may have positive implications for diabetes prevention (Jonsson et al., 2018). Moreover, there are positive effects of rye-based foods on satiety. Regular intake of rye foods has long-term implications for health with other positive effects on inflammation and lowering blood lipids.

2.1.1 Structure and chemical composition of rye

The structure and nutritional composition of rye closely resembles wheat grain (Figure 1) even though the cell walls of rye grain are thicker.

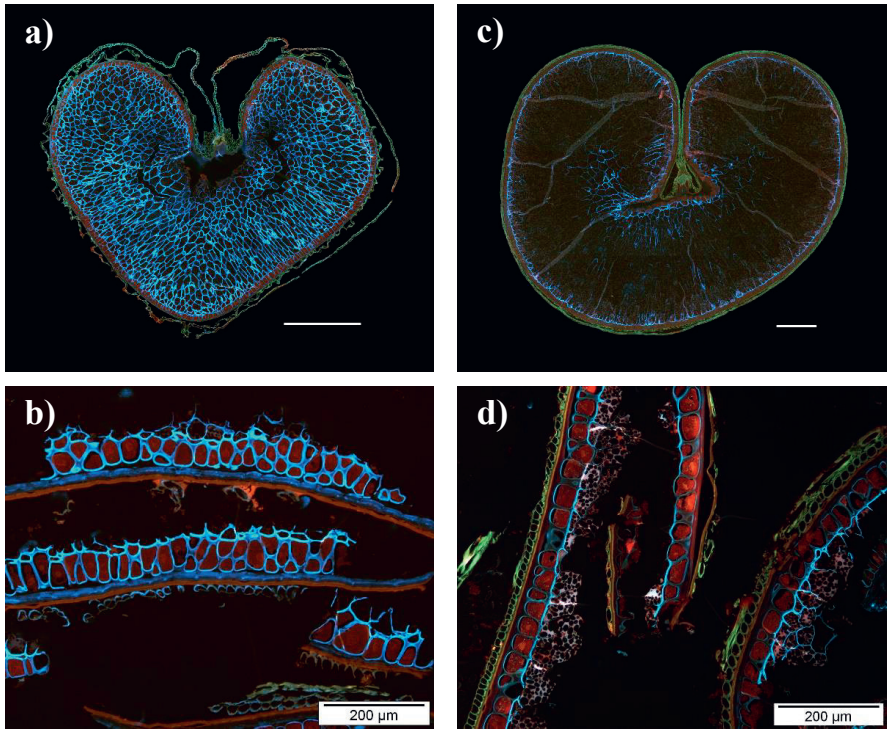

Figure 1. *Distribution of starch (black), cell wall (blue) and protein (red) in the grain and bran of rye (a and b) and wheat (c and d). Calcofluor white and Acid Fuchsin has been used for visualization. The size of the white bars represent 500 µm (a and c) and 200 µm (b and d). Image courtesy of: Ulla Holopainen-Mantila (VTT Technical Research Centre of Finland).*

In both rye and wheat, the cell walls of starchy endosperm are dominated by arabinoxylans (Table 1). In general, rye contains more DF and free sugars but less starch and protein than wheat (Karppinen, 2003). Due to its nutritional and technological functionality, DF is one of the most important component of any cereal grain. DF consists of several carbohydrate polymers linked via β-(1→4)-glycosidic bonds. DF cannot be broken down by host enzymes and thus remains undigested in the small intestine of humans (Singh & Vijay-Kumar, 2020). However, glycoside hydrolase enzymes produced by gut microbes can break down DF glycosidic bonds and thus DF is partially digested in the colon (Bik et al., 2018).

Rye bran (RB) is the by-product of the rye flour industry, and it contains 41–48% DF and is produced in abundance during flour processing. Unlike wheat bran, rye bran contains more β-glucan and fructan but less cellulose (Kamal-Eldin et al., 2009). Rye bran is also rich in starch (13−28%), protein (14−18%), arabinoxylan/pentosan (23%), fructan (7%) and β-Glucan (3.5−5.3%). A small amount of free sugar such as sucrose, maltose with trace amounts of glucose and fructose are also available in rye bran (Kamal-Eldin et al., 2009; Karppinen, 2003; Karppinen et al., 2003). Despite substantial amounts of health promoting DF, rye bran is mostly used as animal feed. However, rye bran could be considered as an important by-product of the cereal food industry due to its high DF and substantial amount of protein.

Table 1. *Chemical composition of rye, wheat, and their brans (g/100 g, dry weight basis).*

Component	Rye	Wheat	Rye bran	Wheat bran
Starch	55–65	67–70	13–28	8–25
Protein	10–15	12–14	14–18	15–17
DF	15–17	10–13	37–48	39–53
Arabinoxylan	8–10	6	20–25	22–30
β-glucan	2–3	1	4–5	1–3
Cellulose	1–3	3	5–7	9–12
Fructan	4–6	1–3	7	3–4
Lignin	1–2	1	4–5	3–5
Fat	2–3	3	4–5	4–6
Ash	2	2	3–7	6–8

Hemery et al. (2007); Kamal-Eldin et al. (2009); Karppinen et al. (2001); Karppinen et al. (2003); Liukkonen et al. (2007); Maes & Delcour, (2002).

2.1.2 Rye in food uses

Rye is mostly consumed as whole grain flour in breads and in other cereal products such as crisp bread and rye flakes. Different rye fractions, e.g., endosperm rye flour and rye bran are also used in a variety of food products including breads, snacks, and breakfast cereals (Heiniö et al., 2003a, b). Rye bran is a DF and protein-rich fraction, which has intense-bitter flavour. The flavour of rye bran becomes more intense during storage without becoming rancid. A typical rye-like flavour is common in rye products, which some consumers may not prefer. Endosperm rye-flour-based products have a mild taste, whereas products with added rye bran have a bitter taste, which is mainly due to phenolic compounds and small molecular weight peptides (Heiniö et al., 2012). The dark colour of rye bran results in a non-appealing colour in rye-based food products, which might have negative impacts on consumer acceptance (Heiniö, 2009; Heiniö, 2014).

Snacks made of rye bran have a grainy flavour, with a strong, bitter aftertaste and flavour, and have coarse texture. It has been shown that the addition of 20% rye bran reduced the brightness and yellowness, but increased the redness compared to oat and wheat bran added extruded corn snacks (Makowska et al., 2015). The same study also showed that corn snacks with 20% added rye bran were less expanded, less crispy, less porous and were less tasty compared to the snacks with added oat and wheat bran. However, increasing the rye bran concentration up to 40% had a less detrimental effect on the structure, texture and colour of the corn snacks compared to wheat and oat bran. Due to the adverse effects of rye bran on the overall quality attributes of the product, various pre-treatments such as sourdough fermentation, germination and milling fractionation are performed on rye fractions to adjust the flavour formation of rye products (Heiniö et al., 2003a, b; Heiniö et al., 2011). For example, mechanically peeled and fermented rye bran (20%) may reduce the colour and flavour intensity of wheat bread with added rye bran (Heiniö et al., 2007). Pre-treatments of rye bran not only improve the flavour but also facilitate higher levels of rye bran incorporation in food products. Processing methods, such as extrusion, also improve the rye flavour in extruded rye snacks. It has been shown in a recent study that the use of fermented rye bran with exopolysaccharide (EPS) producing microorganisms can efficiently improve the expansion and texture of rye flour snacks containing 40% rye bran (Nikinmaa et al., 2017).

2.2 Extrusion processing

Food extrusion processing is a widely used technique to produce cereal foods by means of mechanical shear and heat treatment (Chanvrier et al., 2013; Robin et al., 2012b). Extrusion processing can be done using either single or co-rotating twin screws. A single screw has poor mixing ability, whereas co-rotating twin screws ensure better mixing. During extrusion, feed materials are mixed with water right before a die exit and transformed into a viscoelastic and homogenous melt (Sozer &

Poutanen, 2013). The viscoelastic melt immediately passes through the die exit under high pressure and at a high temperature.

When viscoelastic melt comes out of a small die exit, the rapid pressure and temperature drop cause the melt to expand and the desired shape is produced. Starchy feed materials expand when water vaporizes right after the die exit and form porous and foam like structure. Simultaneous mechanical and chemical changes such as gelatinization and enzyme denaturation of starch and Maillard reactions modify the textural and functional properties of the feed material. In extruded products, starch forms a continuous phase, whereas the protein phase is discontinuous (Sozer & Poutanen, 2013). In DF added extruded products, fibre particles are entrapped in the starch-protein matrices.

The high shear and high temperature during extrusion may cause the protein denaturation and depolymerisation of insoluble DF (IDF). Moreover, enzyme resistant starch may form due to the extreme conditions inside the extruder barrel (Björck et al., 1984). Some of the IDF may also convert into soluble DF (SDF) during extrusion (Singh et al., 2007; Lue et al. 1991). Therefore, the structural properties of extruded cereal foods strongly depend on the extrusion processing parameters as well as the ingredients used in the feed materials.

2.2.1 High fibre extrusion

Expanded and porous structures of the cereal foams are a desired sensory quality for expanded snacks (Guy, 2001). The raw material composition and fibre-flour ratio has profound effects on the properties of extruded products (Altan & Maskan, 2011). High DF extruded products have unpleasing structural and textual properties (Robin et al., 2012c; Sozer & Poutanen, 2013). Bran material and IDF have a poor gas-holding capacity, which restricts the cell expansion (Singh et al., 2007). Moreover, the addition of DF in extruded foods hinders the continuity of food matrices and thus negatively affects both the structural and textural properties. The technological challenges due to the incorporation of DF in extrudates include aspects such as increased hardness and density with poor macro- and microstructural properties (Lue et al. 1991; Sozer & Poutanen 2013). Reduced expansion and crispiness with increased hardness and density were observed in corn-based extrudates when sugar beet (10–30%), soy fibre (10–40 %), and oat, wheat and rye bran (20–40%) was added to the feed (Jin et al. 1995; Lue et al. 1991; Makowska et al., 2015). High DF either ruptures the air cells or prevent the air cells from expanding to their full potential during the nucleation phase, which makes the extrusion processing difficult and challenging. Due to these elements, the incorporation of high DF/bran usually results in less expanded extrudates and leads to small pores and a high density.

Increased amounts of IDF not only cause poor expansion but also increase the cell wall thickness, while reducing the cell size, resulting in a hard, less crispy and dense

product (Guan et al. 2004; Lue et al. 1990; Mendonça et al., 2000; Moore et al., 1990). The negative impacts of IDF on the quality of the products increases with increasing amounts of IDF in the feed. Therefore, the recommended bran addition level typically remains between 10–30% for starch-based extruded products, which reduces the adverse effects of DF on the structural and textural properties. The type of fibre, i.e., IDF and SDF, also influences the properties of the extruded products. For example, IDF such as wheat fibre (95% IDF and 3% SDF) incorporated in corn based extrudates has been found to be hard and less porous, whereas SDF, such as pectin, resulted in less hard and porous extrudates (Yanniotis et al., 2007). Although both the wheat fibre and pectin reduced the expansion, pectin was found to improve the textural properties. The number of cells and the cell size were reduced with the addition wheat fibre, while they were not affected in the products with added pectin (Yanniotis et al., 2007). Therefore, the properties of extruded products are crucially influenced by the incorporation of IDF and thus require some technological solutions to overcome the negative impacts.

2.2.2 Effects of dietary fibre on structure formation

There are two types of extruded solid foams: one has an open structure (e.g. extruded puffs) and the other has a closed structure (e.g. extruded flakes). Open solid foams consist of a porous structure, in which pores are interconnected by thin cell walls. Open solid foams are usually brittle and less dense compared to closed solid foams (Dogan et al., 2008). Expansion is one of the most important quality parameters of palatable extruded foods. The expansion rate of the extruded products are influenced by their density and microstructure (e.g. porosity, cell diameter, and cell wall thickness). It has been reported in several studies that the macro and micro structures of the extrudates are impeded by the addition of insoluble fibre (Guan et al., 2004; Lue et al., 1990; Mendonça et al., 2000; Moore et al., 1990; Robin et al., 2011a, b).

Increasing the DF content by adding different fibre sources e.g. wheat, oat and sugar beet fibre reduces the expansion of corn extrudates. In earlier work, increased amounts of soy fibre in corn extrudates resulted in less expanded extrudates with smaller air cells and thicker cell walls (Jin et al., 1995). In other studies, wheat bran and soybean hull additions resulted in the reduced expansion and increased density of wheat and corn based extrudates, respectively (Carvalho et al., 2010; Robin et al., 2011a). The reduction in the number of air cells, smaller air cell size, and changes in the air cell distribution could be explained by the inverse relationship between expansion and the DF content (Lue et al., 1990; 1991). A higher concentration of bran in extruded products results in higher amounts of free water and thus may reduce both the glass transition and melting temperature of starch, which may reduce the starch transformation and in turn the expansion rate (Robin et al., 2011c). Moreover, the elastic properties of the starchy melt is reduced due to the low adhesion properties between starch and bran particles, which causes poor expansion (Robin et al., 2012a, c). It has been shown that the reduced expansion of extruded products is accompanied

by the use of coarse bran/DF material or whole grain (Lue et al., 1991; Mathew et al., 1999). However, the adverse effects of coarse bran or DF materials on expansion could be reduced by milling the bran or DF material into fine particles.

The structural properties of the extrudate also depend on the concentration of DF, protein and fat contents in the feed material (Bhattacharya & Prakash, 1994). Under the same processing conditions, increased amounts of bran (wheat bran: 12.6–24.4 % DF) in wheat-flour-based extrudates were reported to reduce the expansion (Robin et al., 2011b). Additionally, increased amounts of oat and wheat bran have been shown to reduce the expansion of wheat and whole-wheat flour extrudates (Chassagne-Berces et al., 2011). Therefore, in earlier works, typically 10–30% of cereal bran was used to avoid the adverse effects of cereal bran on the quality attributes of extruded products (Robin et al., 2012c; Sozer & Poutanen, 2013).

The type of the fibre source (IDF vs SDF) also affects the structural properties, e.g. the expansion and density of the extruded products, but the effect can be different even at same addition levels. In one test, the addition of SDF such as 15% inulin or guar gum did not alter the expansion, while a similar amount of IDF such as wheat bran reduced expansion (Robin et al., 2012b). However, both the IDF and SDF increase the bulk density and reduce the porosity. Under the same extrusion conditions, increased amounts of DF content (by adding 32% wheat and oat bran) have been found to reduce the porosity and the average cell size of wheat and corn extrudates regardless of the fibre sources (Chanvrier et al., 2013; 2014). On the other hand, an increase in porosity (82−92% vs 59−90%) and average cell size (600−1200 μm vs 150−600 μm) has been reported for whole wheat compared to corn based extrudates (Chanvrier et al., 2014). The impacts of fibre addition in whole-wheat extrudates have been found to be less pronounced compared to corn based extrudates. However, for both whole wheat and corn extrudates, it has also been found that the cell wall thickness is not affected by fibre addition (Chanvrier et al., 2014).

In a study by Parada et al. (2011) microstructural properties of the extrudates were found to be influenced by both the DF source (potato, rice, wheat, and corn) and the addition level (0–10%). The study showed that the amount of fibre affected the cell separation and the cell wall thickness in all extruded products. An increase in fibre amount increased the number of air cells in all the tested extruded products, but the degree of anisotropy decreased for potato, rice, and wheat extrudates, whereas no effect was observed for corn extrudates in terms of the degree of anisotropy. In another study, the addition of 10% oat bran concentrate into oat endosperm flour increased the cell wall thickness (340−420 vs. 320 μm) compared to 100% endosperm flour extrudates (Sibakov et al., 2015). In the same study, finely milled (32 μm) oat bran extrudates were more porous compared to coarse (213 μm) oat bran. Water insoluble oat bran resulted in an increased number of small pores with thicker cell walls compared to water soluble oat bran. Therefore, the solubility of DF is crucial to determine how fibre particles will affect the microstructural properties of extrudates.

2.2.3 Effects of dietary fibre on texture formation

High-fibre extruded snack products are gaining consumer interest due to their positive health benefits (Robin et al., 2012b). It has been found that the raw material composition, matrix architecture and homogeneity of the solid matrix determine the texture of the extruded products (Sozer et al., 2011b). For this reason, quite a lot of research has looked at aspects that influence the texture of extruded food products. A study by Robin et al. (2011a) found that the hardness of extrudates increased when wheat bran was added to wheat based extrudates. The effect of the bran addition on the textural properties was found to depend on the particle size of the bran material. In a variety of studies, coarse bran has been found to alter the textural properties of extrudates. For example, a higher percentage of coarse bran in starchy matrices was found to increase the hardness and reduce the crispiness (Brennan et al., 2008a; Makowska et al., 2015; Mendonça et al., 2000; Moore et al., 1990; Yanniotis et al., 2007). It has been suggested that crispier and less hard extrudates could be achieved either by using fine bran or by reducing the bran addition level (Lue et al., 1991; Mathew et al., 1999). However, in one study by Robin et al. (2011a) the incorporation of finely milled wheat bran increased the number of small cells in the extrudate matrices, which required more force to rupture. A similar effect was observed with wheat and oat bran in a study by Chassagne-Berces et al. (2011), in which the addition of fine particle sized bran resulted in hard and less crispy extrudates. The extrudate's microstructure (e.g. the average cell diameter, cell wall thickness and cell number density) has a direct impact on the mechanical properties (e.g. compression modulus, crushing force and crispiness work). The microstructural properties have been shown to have a good correlation ($r = 0.48$ to 0.81) with the mechanical properties (Agbisit et al., 2007).

Extrusion process parameters such as the screw speed and water feed have been found to determine the extrudate's interior architecture, consisting of closed air cells of varying shapes. For example, increasing the screw speed has been found to increase the cell diameter and decreases the number of cells (Trater et al., 2005). Additionally, the cell diameter and cell wall thickness have a significant influence on mechanical properties. For example, in a study by Agbisit et al. (2007), the cell diameter was shown to have a negative correlation with hardness ($r = -0.79$) and crispiness work ($r = -0.81$). It was also found by Agbisit et al. (2007) that extrudates with large cells with thinner cell walls had a lower compression modulus and required low crushing force compared to the extrudates with small cell with thicker cell walls. It has also been found that extrudates with similar expansion properties might be different in terms of their pore size distribution (Horvat et al., 2014). However, the porosity and expansion rate of the extrudates influence the textural properties and thus could be used to predict sensory quality.

Robin et al. (2010; 2011a, b, c; 2012a, b, c) extensively studied the microstructural and textural properties of wheat bran supplemented extrudates. They observed that

expanded extrudates with fewer air cells are usually less hard and require a low force to rupture. Higher amounts of insoluble fibre in starchy extrudates give rise to early bursting air cells, and thus result in less expanded, dense structures with a higher number of small cells.

2.3 Strategies to improve structure and texture in high fibre extrusion

2.3.1 Optimization of process parameters

Extrusion processing is a commonly used technology to incorporate DF into snack products. Changing the processing parameters or making small changes in the core composition of the raw materials may result in large changes in the shape and quality of the extrudates. High fibre extrusion is challenging, but properly selected extrusion process parameters and raw material compositions could help to produce extrudates with desirable structures and textures (Zarzycki & Rzedzicki, 2009). Furthermore, changes in the starch-fibre ratio could improve the structural and textural properties. For example, expanded and softer extrudates can be obtained by increasing the amount of starch (Stojceska et al., 2008a).

Extrusion process parameters (e.g. screw speed, feed rate, water content of the feed and temperature profile) have significant effects on the physical properties of the extrudates (Rzedzicki et al., 2000). A high screw speed increases the shear force inside the extruder barrel by altering the mass temperature, torque, and pressure, and thus influences the products' expansion and texture (Sokhey et al., 1994). The extrusion temperature plays a significant role concerning the extrudate's structure. A high extrusion temperature reduces the melt viscosity, and a low viscous melt triggers the collapse of the air cells under high-pressure, and finally results in less expanded extrudates (Moraru and Kokini, 2003). It has been reported in numerous studies that the combination of a high screw speed and a low water content of the feed could facilitate the production of good quality extrudates with high DF (Ainsworth et al., 2007; Robin et al., 2011b; Stojceska et al., 2008b). For example, Robin et al. (2011b) found that a combination of a high screw speed, high temperature and low water content resulted in improved structures of wheat based extrudates containing 12.6–24.4% wheat bran. The water addition to the feed material can be done either by adding it directly to the extruder barrel or by pre-conditioning before feeding the material into the extruder. Pre-conditioning has been found to improve the product quality, which enhances the extrusion process through a longer equilibration time and higher moisture content retention (Riaz, 2001). However, a low water content is preferable to avoid an undesirable hard and less crispy texture (Yağcı et al., 2012).

The extrusion process conditions also play a significant role in determining the products' nutritional quality. Singh et al. (2007) studied this and found that a moderate

high-water feed, i.e. higher than 15%, and a low temperature, i.e. less than 200 °C, could be used to improve the nutritional quality of the products. However, longer residence times should be avoided since a longer residence time may cause some nutrient loss. Therefore, carefully choosing the optimal processing conditions will not only improve the structural and textural properties but it will also help to retain nutritional quality. In this regard, the determination of the feed material composition, proper formulation and selecting the right processing conditions with an optimized hydration regimen is needed to improve the overall quality of extrudates.

2.3.2 The role of particle size reduction

The role of particle size reduction of the flour and/or bran in the extrusion process has been studied by various researchers (Lue et al., 1990, 1991; Guan et al., 2004; Al-Rabadi et al., 2011a, b; Sibakov et al., 2015). The expansion of starchy extruded products is associated with starch gelatinization. The use of coarse particles in the feed during extrusion lowers the specific mechanical energy and barrel temperature, and thus results in reduced gelatinization, and in turn reduces expansion in the final products. Coarse fibre particles also interfere with the air cell growth by rupturing them before optimal expansion occurs (Lue et al., 1991). Moreover, due to the short residence time in the extruder barrel, water is unable to penetrate coarse particles, thus starch granules remain in a hard state and this results in an incomplete plasticization (Zhang & Hoseney, 1998). A lower degree of starch gelatinization may occur during extrusion due to incomplete plasticization, which could also result in poor expansion (Al-Rabadi et al., 2011). On the other hand, due to the high water-binding capacity, fine fibre particles provide more nucleation sites for water vapour to develop when the material exits the die. Therefore, the air cells expand due to the vaporization force, which thus leads to a higher number of expanded cells (Lue et al., 1991). It has also been shown that particle size reduction of bran has an impact on the hydration capacity of the mass during extrusion and in its associated melt rheology (Sozer & Poutanen, 2013). Extrudates made with fine particle sized corn flour have been found to expand more than their coarse counterparts (Garber et al., 1997; Mathew et al., 1999). Similar results were reported for corn extrudates with added sugar beet fibre (Lue et al., 1991). However, opposite results were reported for finely milled corn flour extrudates (Carvalho et al., 2010). Finely milled corn flour was found to heat up more rapidly and therefore reached the melt transition temperature earlier than coarse corn flour, which resulted in a low viscous melt and leads to a less expanded extrudates (Carvalho et al., 2010). Similar results were reported for oat bran (10%, 32 μm) supplemented defatted oat flour (213 μm) extrudates, where a particle size reduction did not show any significant increase in expansion (Sibakov et al., 2015). Robin et al. (2011a) showed that there was no significant effect of particle size reduction of wheat bran (from 317 μm to 224 μm) on the quality of wheat flour based extrudates. However, in their study only 30% reduction of the particle size was not sufficient at low addition level to show any observable changes in the structure and texture of extrudates.

The particle size of the feed not only affects the structural properties but also influences textural characteristics. However, the effect may vary depending on the flour-fibre ratio (i.e. fibre concentration) and on the feed material type (Garber et al. 1997; Guy, 1994; Mathew et al., 1999; Onwulata & Konstance 2006; Robin et al., 2011a). It has been shown in previous studies that particle size reduction of the feed material (flour, grit, and bran) not only improves the structure but also produces softer extrudates and a crispy texture (Al-Rabadi et al., 2011; Lue et al. 1991; Mathew et al. 1999; Zhang & Hoseney 1998). A recent study showed that the expansion of rye extrudates improved significantly when 20% finely milled wheat bran (84 μm) was added compared to coarse wheat bran (702 μm), but there was not any improvement in the textural properties (Santala et al., 2014). A limited number of studies (e.g. Alam, 2012; Al-Rabadi et al., 2011; Guan et al. 2004; Lue et al., 1990; 1991, Robin et al., 2011a, b) have focused on how the microstructural and textural properties of high fibre extrudates were influenced by the particle size reduction of the feed materials.

However, to date there are no studies available that investigate the relationship between particle size reduction and crispiness of the extrudates, which is considered one of the key quality parameters of extrudates for acceptance from consumers. Therefore, more research is needed which will not only study the effect of particle size reduction on the expansion properties, but which also includes microstructural (porosity and cell wall thickness) and textural (hardness and crispiness) properties.

2.3.3 Bran fermentation prior to extrusion

The structural and textural properties as well as the flavour of high DF foods (e.g. wheat and rye bran enriched bread) was shown to be improved by bran fermentation with lactic acid bacteria (LAB) and yeast (Coda et al., 2014; Katina et al., 2012; Salmenkallio-Marttila et al., 2001). Although the fermentation of wheat and rye bran has been shown to have technological advantages in baked products, its use in extruded products remains less studied (Nikinmaa et al., 2017). Exopolysaccharides producing LAB increase the viscosity of the ferment due to the hydrocolloid nature of the polysaccharides. Therefore, *in situ* produced EPS is an industrially interesting option to be used as a natural hydrocolloid in cereal-based foods (Galle & Arendt, 2014; Shukla et al., 2014; Zannini et al., 2016). Some LAB such as *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* are able to produce an inducible dextransucrase enzyme and thus have the potential to generate dextran $(C_6H_{10}O_5)n$ and fructose $(nC_6H_{12}O_6)$ from sucrose $(nC_{12}H_{22}O_{11})$ (Lacaze et al., 2007; Maina et al., 2008; Zannini et al., 2016).

Dextrans are a large group of α glucans with a 50% α-(1→6)-glycosidic linkage in the main polymeric backbone accompanied by an α-(1→2)-, α-(1→3)-, and/or α-(1→4)-branched linkage (Ahmed et al., 2012; Bounaix et al., 2010; Lynch et al., 2018). During the enzymic reaction, dextran is attached to the enzyme at the reduction end, whereas glucose units are linked to the reducing end through the interpolation

between the growing dextran chain and dextransucrase (Lacaze et al., 2007). A high sucrose content enhances higher dextran production, but an increased amount of sucrose may result in low molecular weight dextran (< 10^6 Da), which has a higher degree of branching (Kim et al., 2003). High molecular weight dextrans with less branching is more soluble in nature and thus increases the technological benefits and is more efficient in improving bread volume than high molecular weight dextrans with more branching (Bounaix et al., 2010; Galle & Arendt, 2014; Shukla et al., 2014; Zannini et a., 2016).

Lacaze et al. (2007) found that high molecular weight dextrans are formed at a lower sucrose concentration (10%) in the system. Moreover, a high initial sucrose content may result in abundant residual fructose in a fermented product, which may cause excessive browning and texture changes through the Maillard reaction (Kajala et al., 2015; Lacaze et al., 2007). The taste of cereal products is negatively influenced by acidity. Therefore, to achieve maximum technological benefits, a high dextran content with minimal acidification is needed (Kajala et al., 2015). *Weissella confusa* have been shown to produce less organic acid during fermentation compared to other sourdough LAB (Katina et al., 2009), whereas dextran produced by *in situ* fermentation masks the sourness associated with the acidification (Galle et al., 2012). *Weissella confusa* are LAB which have the potential to produce linear dextran with a molecular weight of around 1.8×10^7 g/mol with a 97% α-(1→6) and 3% α-(1→3)-linkage (Bounaix et al., 2009; Kajala et al., 2015; Maina et al., 2008; Shukla et al., 2014). It has been shown that high molecular weight dextran producing stains result in less organic acids and deficiencies in transforming fructose into acetate and mannitol. When other strong substrate acceptor molecules for dextransucrase such as maltose or isomaltose are present in the reaction mixture along with sucrose, a low molecular weight oligosaccharide may produce (Bounaix et al 2010; Galle & Arendt, 2014; Lacaze et al., 2007; Zannini et al., 2016). This synthesis of oligosaccharides may lead to a decrease in the formation of high molecular weight dextrans (Lynch et al., 2018; Zannini et al., 2016). However, EPS from *Weissella confusa* contains only glucose and therefore the EPS produced by these strains are only dextran (Bounaix et al., 2009; Maina et al., 2008), which has an average molecular weight of 1.8×10^7 g/mol with about 2.4−3.3% α-(1→3)-linked branches.

For the reasons mentioned above, dextran produced by *Weissella confusa* could be a suitable alternative to widely used dextrans (with about 4−5% α-(1→3)-linkages and molecular weights of $1.5−2 \times 10^6$ g/mol) from commercially available *Leuconostoc mesenteroides* (Ahmed et al., 2012; Bounaix et al., 2009; Lacaze et al., 2007; Maina et al., 2008). Externally added dextran does not have similar technological benefits compared to dextrans produced during *in situ* fermentation. In a study by Nikinmaa et al. (2017) no improvements were found in the structural properties of rye-based extrudates with added rye bran (20%) supplemented with 5% commercial dextran compared to untreated rye bran extrudates. Brandt et al. (2003) showed that externally added polysaccharides were not as effective in improving bread structure compared to polysaccharides produced during *in situ* fermentation. It has been shown that rye bran

fermented with EPS producing *Weissella confusa* improved the structural and textural properties of extrudates even at higher bran addition levels (Nikinmaa et al., 2017).

2.4 Structure breakdown during oral processing

2.4.1 Mastication process

Mastication is the process of systematic mechanical breakdown of food followed by the formation of a bolus (Bornhorst & Singh, 2012). In the mouth, a food product disintegrates into smaller fragments through complex mechanical and chemical transformations (Chen, 2015). Smaller fragments of the food agglomerate and mix with saliva to form a lubricated and cohesive mass known as a bolus (Bornhorst & Singh, 2012; Jalabert-Malbos et al., 2007; Le Bleis et al., 2013). The secretion of saliva (ml/min) during mastication does not depends on the eaten foods and remains fairly stable regardless of the type of food (Gavião et al., 2004). However, the amount of saliva that mixes with the masticated foods still may vary since foods with different compositions and structures require different mastication times. Foods which have longer mastication times will be mixed with more saliva (Gavião et al., 2004). Saliva contains mostly water (approx. 99%) and many other important substances such as electrolytes and mucin, salivary α-amylase, maltotriose, and α-limit dextrins (Butterworth et al., 2011; Moska & Chen, 2017). Salivary α-amylase hydrolyses the α-(1→4)- glycosidic bonds of starch into maltose and initiates the starch digestion process. Starchy food digestion starts immediately once the food particles are lubricated with saliva during bolus formation.

Mastication and bolus formation vary between subjects and between products (Tournier et al., 2014). Food products' structural (i.e. expansion, porosity, and density) and textural (i.e. hardness and crispiness) properties are assumed to have a great influence on mastication and physiological responses (Kong & Singh, 2008; Turgeon & Rioux, 2011). The hardness of food products is inversely related to the mastication time and the rate of fracture events (i.e. food breakdown) which occur during mastication (Agrawal et al., 1997). For example, the mastication time required for mouthfuls (4 g) of carrots, Emmental cheese and egg whites was found by Jalabert-Malbos et al. (2007) to be 19, 15 and 8 seconds, respectively, whereas the mastication was approximately 34 cycles for carrots, 24 for Emmental and 14 for egg white

Cereals foods have a considerable amount of starch and therefore the incorporation of saliva in the bolus during mastication is an important determinant for the breakdown of starch (Bornhorst & Singh, 2012). For instance, 25–50% of pasta and wheat bread starch have been found to be hydrolysed during mastication with the activity of salivary α-amylase (Hoebler et al., 1998). The viscosity of a starchy foods bolus is also reduced by the action of salivary α-amylase during mastication (Kong & Singh, 2008).

The hardness and the elastic behaviour of food influence the breakdown of food materials (Bornhorst & Singh, 2012). The type of the food determines the bolus particle size distribution after mastication (Bornhorst & Singh, 2012). A median particle size of a cereal food after mastication depends on the initial structure. For example, in studies, the particle size distribution was 1.5 mm for wheat flakes and 1.9 mm for wheat bread, whereas cooked spaghetti was swallowed with a particle size ranging between 2.5–30 mm (Hoebler et al., 1998; Le Bleis et al., 2016; Peyron et al., 2011).

Although it is still not well understood, how food properties affect mastication, it is believed that expanded and crispy products require a lesser extent of mastication than hard and dense extrudates (Pentikäinen et al., 2017). It has been shown that food hardness has an increasing effect on the number of chews for biscuits, apples, and bananas even though the total chewing time remains unaffected by the food texture (Hiiemae et al., 1996). Dry food needs more time to mix with sufficient quantities of saliva for lubrication. For example, foods such as biscuits, which are dry and hard, require a larger number of chews before swallow, but softer food such as bananas require a lower number of chews. Hiiemae et al. (1996) also observed that increased food hardness tends to result in a reduced bite size. For example, the mean bite size (weights) for biscuits (hard), apples (intermediate) and bananas (soft) were 3, 7 and 13 g, respectively. Therefore, the hardness of the food has a significant role on the overall mastication process. The mastication action and pattern of an individual human depends on some physiological characteristics such as gender, age, personality type, dentition status, facial anatomy and finally on the time of day. Moreover, food properties such as structure, texture, water and fat content, as well as the food portion size also play a significant role in mastication and bolus formation (Bornhorst & Singh, 2012).

2.4.2 The effects of the bolus particle size

Simultaneous processes of food breakdown occur during mastication followed by agglomeration and lubrication for preparing a bolus. Bolus particles once they have achieved the appropriate size and have been sufficiently lubricated by saliva proceed to the stomach for further digestion. Several factors such as the moisture, fat content, and texture of food determine the particle disintegration, saliva secretion and in turn bolus formation (Bornhorst & Singh, 2012). However, it has been found that the structure and texture of the food dictates the particle size distribution (Hoebler et al., 2000; Jalabert-Malbos et al., 2007). Food products with a hard and less crispy texture result in larger particles in the bolus, while soft and crispy products disintegrate more easily thus producing smaller particles (Pangborn & Lundgren, 1977).

The reduction of the particle size of the eaten food is required for bolus formation since small fragments can be packed together tightly by compression between the palate of the mouth and the tongue (Prinz & Lucas, 1997). During mastication, foods

should have achieved a particle size of less than 2 mm before swallowing (Jalabert-Malbos et al., 2007). However, foods which have a soft texture can also be swallowed easily in larger particles and therefore the critical particle size in the swallowable bolus may vary between 1 and 3 mm depending on the food texture (Le bleis et al., 2016). After swallowing the bolus is conveyed to the stomach for further digestion. In the stomach, food particles mix with gastric juice (a mixture of inorganic salts, enzymes, mucus, intrinsic factors, and hydrochloric acid) and further break down with the help of gastric secretion and by peristaltic contraction of the oesophagus (Bornhorst & Singh, 2014¸ Bornhorst et al., 2016). Salivary α-amylase become inactive in the stomach due to the gradual decrease of the pH, while the activity of pepsin and other gastric enzymes starts. In this phase, hydrochloric acid and enzymes hydrolyses the bolus and form a semi-fluid mass known as chyme. Partly digested chyme contains smaller particles less than 1-2 mm and this is released to the duodenum through the pyloric valve (Jalabert-Malbos et al., 2007; Kong & Singh, 2008; Thomas, 2006). However, the literature is lacking research on how the disintegration and bolus formation of extruded foams is affected by their structural and textural properties and is mainly focused on commercial flakes made from refined flour (Hedjazi et al., 2013; 2014; Yven et al., 2010).

2.5 Starch digestibility

2.5.1 Starch digestion process

Starch is the storage carbohydrate of plants, which consists of linear amylose and branched amylopectin glucose polymers (Singh et al., 2010). Starch is one of the major sources (20−50%) of energy intake in human diets other than protein and fat (Bohn et al., 2018). Starch in foods can be classified as slowly and rapidly digestible starch, while another type remains undigested and is termed as resistant starch (Englyst et al., 1992). Salivary α-amylase initiates starch digestion in the mouth by breaking down complex carbohydrates and produces maltose, maltotriose and α-limit dextrins by cleaving the α-(1→4)-glycosidic bond (Robyt & French, 1970). Salivary α-amylase is most active at pH 6.8 and the optimum pH for enzymatic activity ranges between pH 6 to 7. Below and above this range the enzymes become denatured and thus enzymatic activity is reduced. The acidic environment of the stomach causes the salivary amylase to denature and stops the action of the α-amylase. Therefore, salivary α-amylase does not function once it enters the stomach due to the acidity (pH 3.3−3.8) caused by the gastric acid (Pedersen et al., 2002). The small intestine plays an important role in digestion. Glucose is produced to be absorbed in the small intestine through further hydrolyzation of maltose, maltotriose and α-limit dextrins by brush-border enzymes i.e. amyloglucosidase (Rosenblum et al., 1988). Therefore, some researchers believe that saliva should not be considered to play a major role in carbohydrate digestion.

Starch digestion in the human body can be determined *in vivo* by monitoring changes in blood glucose concentrations. The Glycaemic Index (GI) has become the most popular tool to compare postprandial glycaemic responses since the concept of the GI was developed in 1980s (Bohn et al., 2018; Jenkins et al., 1981). The GI is defined by the incremental area under the blood glucose response curve (AUC) after a food eaten containing 50 g of available carbohydrate is compared to the AUC of a reference food (e.g., refined wheat bread and glucose solutions) containing equivalent amounts of available carbohydrate (ISO, 2010). Foods with a low GI have been suggested to help reduce the risk of coronary heart disease, diabetes, and obesity (Blaak et al., 2012). Starch-based extruded products are considered high GI foods due to their high GI rating (> 70) and therefore they are reported to be associated with the above mentioned diseases (Livesey et al., 2008). As a result, consumer demand is growing for low GI foods and thus opting for healthier choices, which in turn is motivating food manufacturers to design high DF snacks. Although internationally accepted protocols exist for measuring the GI of foods (FAO/WHO, 1998), several methodological challenges including the physiology of the subjects, method of blood sampling and other macronutrients than carbohydrates in the test foods result in significant variation in the measured GI (Hätönen, 2014). Due to the complexity of *in vivo* methods of determining glycaemic responses, various *in vitro* techniques have been developed to predict different phases of human digestion (Bohn et al., 2018). *In vitro* methods are performed by mixing α-amylase enzyme with ground food products at various pH levels according to different phases in the gastrointestinal tract. The hydrolysis index of the starch-based food is a widely used *in vitro* method to predict metabolic responses (Grandfeldt et al., 1992).

2.5.2 Factors influencing starch digestion

In addition to the degree of crystallinity of starch granules, the amylose-amylopectin ratio is one of the main factors relevant to starch digestibility. Amylose is less susceptible to hydrolysis than amylopectin and therefore starch rich in amylose is more resistant to hydrolysis (Singh et al., 2010). The size and surface area of the starch granule is also important in determining the starch digestibility. Small starch granules are digested rapidly due to their higher specific surface area compared to large granules. The accessibility of digestive enzymes to starch may be restricted by proteins, which cover starch granules and restricts their gelatinization (Singh et al., 2010). Complex formation with fatty acids may also restrict starch digestibility by reducing the solubility of the starch and increasing the gelatinization temperature.

Processing that disrupts the integrity of starch granules may also influence starch digestibility, for example native starch with intact grains is extremely resistant to hydrolysis, whereas milling/grinding increase the surface area and makes the starch molecules more susceptible to enzyme hydrolysis (Mishra et a., 2012; Singh et al., 2010). Starch become more susceptible to enzymic reactions when gelatinized under heat treatment in the presence of water, for example during extrusion (Schirmer et al.,

2015). Hence, the starch-water ratio determines the extent of starch gelatinization and therefore becomes an important determinant of the starch digestibility rate of processed food (Singh et al., 2010).

The food structure formed during processing influences the accessibility of digestive enzymes to the starch present in the food matrix (Mishra et al. 2012). Foods with open porous structures (e.g. bread, cakes, and expanded extruded products) have a high internal surface area and are thus more available to enzymic action than foods with dense structures (e.g. pasta). Foods which have a higher density are not easily accessible by digestive fluids; enzymes will only be exposed to the starch of dense food by eroding superficial layers. Therefore, starch digestibility of dense food depends on the surface area of the bolus particles.

The viscosity of the food digesta is another factor that controls starch digestibility in the stomach. High viscosity of the food digesta may restrict/slow down the permeation of gastric juices into food bolus phase (Lentle & Janssen, 2010). Slower permeation of the gastric juices slows down the digestion of the food bolus and the bolus is retained for longer period of time in the gastric lumen. The disintegration of the food, solubilisation of the food components, hydration, as well as swelling capacity determine the viscosity of food digesta (Lentle & Janssen, 2008). For instance, foods with added SDF (such as oat bran and guar gum) have the ability to increase their viscosity during digestion (Dikeman et al., 2006). Viscosity formation due to the solubilisation of DF also depends on the form of DF eaten for example, whether it is eaten as an extract (active ingredients in concentrated form) or as part of the food.

2.5.3 Types of food and structure breakdown

The breakdown of foods in the stomach may vary depending on the initial food structures and product category (Bornhorst & Singh, 2014). For instance, after gastric digestion, the highest fraction of large particles was found to remain in rye crispbread followed by sourdough fermented rye bread, whereas extruded rye snacks disintegrated to smaller particles compared to both bread types (Johansson et al., 2018). The disintegration of the food structure is not only dependent on the product category but also on differences within categories. For example, rye bread has been found to contain a higher share of large particles after gastric digestion compared to wheat bread. Similarly, brown rice containing bran has been found to remain in larger particles than white rice without bran (Bornhorst et al., 2013; Johansson et al., 2018; Nordlund et al., 2016).

The starch digestibility of extruded foods depends on many factors including the feed material composition, processing method, product type, as well as the structure, and textural properties (Singh et al., 2013). It is thus crucial to understand how the digestibility of starch is affected by the structural and textural properties of extruded foods containing high amounts of DF. The addition of different fibre sources (e.g. guar

gum, inulin and fenugreek polysaccharide) into extruded products reduced up to 5-15% of both *in vivo* glycaemic responses and the *in vitro* starch digestibility rate (Brennan et al., 2008b; Shirani & Ganesharanee, 2009). Therefore, it is believed that the addition of rye bran into extrudates may have a similar effect on the *in vitro* starch digestibility (Sozer & Poutanen, 2013). Food macro- and microstructural properties of starchy foods have been shown to be correlated with disintegration, bolus formation, gastric emptying, and glucose responses. However, when gastric digestion was tested in *in vivo* human trials using non-invasive methods to measure gastric emptying or glucose responses after eaten, the precise relationship between the food structure-texture and its bolus disintegration was not found (Bornhorst & Singh, 2012). Therefore, *in vitro* methods such as starch hydrolysis index could be utilized for this purpose (Bornhorst & Singh, 2012).

3 Aims of the study

The aim this work was to elucidate how bran addition and bran pre-treatments influence the structure and texture of extruded products and further oral breakdown and starch digestibility. The specific aims were:

I. to understand the main structural and mechanical features of extruded rye products

II. to investigate how extrusion processing and bran modification (e.g., particle size reduction and fermentation) affect the structure formation of high fibre rye extrudates

III. to focus on the role of structure breakdown in *in vitro* starch digestibility

4 Materials and methods

4.1 Feed material preparation

Commercial rye bran (D_{50}: 750–1250 µm, Figure 2) was collected from Fazer Mill and Mixes (Lahti, Finland). Rye bran in three different particles sizes (D_{50} coarse: 440 µm, medium: 143 µm and fine: 28 µm) was obtained (Publications 1−2) by milling commercial (native) rye bran at the VTT Technical Research Centre of Finland (Espoo, Finland). Coarse and medium rye bran was prepared by milling native rye bran in an Alpine fine impact mill at 17,800 rpm (100 UPZ, Hosokawa Alpine, Augsburg, Germany). Coarse rye bran was obtained by milling native rye bran twice with 1 mm sieve and medium rye bran twice with 0.3 mm sieve. Fine rye bran was prepared using a G-55 Turborotor Mill (Görgens Mahltechnik GhmH, Dormagen, Germany) with 60 Hz motor cycles and with an average feed rate of 30 kg/h. Rye bran of 3-different particle sizes was extruded in Publication 1 without any added starch or flour to understand the basis of rye bran extrusion.

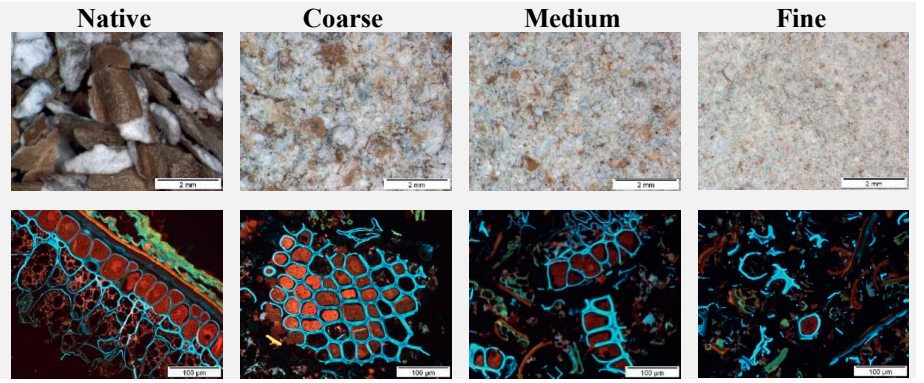

Figure 2. *The distribution of starch (black), cell wall (blue) and protein (red) in the native, coarse, medium and fine rye bran. Calcofluor white and Acid Fuchsin has been used for visualization. The size of the white bars represent 2 mm (first row) and 100 µm (second row).*

Rye endosperm flour (RF, 85–97% < 132 µm) was used as a base material (Publications 2−4) and was obtained from Helsingin Mylly Oy (Järvenpää, Finland). RF has 75% starch, 7% total dietary fibre (TDF) and 6% protein. Waxy corn starch (Roquette Ltd., France) with 97% amylopectin was used to prepare a starch-flour mix (Publication 2). The starch-flour mix was prepared by adding 30% starch to 70% RF using a spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany). Coarse or fine-particle sized rye bran was used together with a starch-flour mixture. The bran incorporation was categorized as high (30% bran: 74% starch and 12.6% TDF) and low (15% bran: 82% starch and 8.2% TDF) DF feed material. A spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany) was used to prepare the final blend of starch-flour-bran.

Commercial rye bran (Publication 3) was obtained from Fazer Mills and Mixes Ltd. (Lahti, Finland), which was further milled to obtain a particle size of D_{50} 24 µm. Ultra-fine Ceren Miller DAU (Masuko Sangyo Ltd. Co., Kawaguchi, Japan) grinding equipment was used to grind the rye bran. Directly expanded puffs and flakes were prepared either using 100% RF or with the addition of 10% rye bran to increase the fibre content. In the dry mix of all recipes 0.8% salt was added. The mixing of rye flour and rye bran was performed using a spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany).

Native rye bran (Publication 4) was prepared through repeated milling and air classification of native rye kernels (Jalon Mylly, Kouvola, Finland) using a 100 UPZ-lb fine impact mill (Hosokawa Alpine AG, Germany) with stainless steel pin discs (17 800 rpm), followed by air classification (speed 3500 rpm, airflow 220 $m^3\ h^{-1}$, feed rate 50 kg h^{-1}) (British Rema Minisplit, Chesterfield, UK) to obtain a particle size of D_{50} 361 µm.

Fermented rye bran (FB) was obtained by fermentation (Publication 4) of native rye bran using EPS-producing *Weissella confusa* (VTT E-133279), which was collected from the VTT Culture Collection (VTT Technical Research Centre of Finland, Espoo). The fermentation of the bran material was performed with a bran:water ratio of 22:78 using a protocol published by Nikinmaa et al. (2017). Bran fermentation was carried out in a total weight of e.g., 500 g with or without (control rye bran) sucrose supplementation. The fermented samples contained 110 g (22% *w/w*) rye bran and 390 g (78% *w/w*) sterile tap water including the cell suspension. Sucrose was used as a substrate for dextran production thus 10% (i.e. 11g) of the bran was replaced with sucrose. A beaker (1 L) containing sucrose, water and rye bran was incubated at 25 °C for 20 hours. Fermentation of the bran materials was performed in duplicate. A Christ Epsilon 2–25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) was used to dry the fermented bran. Dried rye bran was ground using a 100 UPZ-lb fine impact mill (Hosokawa Alpine AG, Germany) at 17,800 rpm with stainless steel pin discs.

4.2 Particle size analysis

The particle size of the raw materials (Publications 1−4) was determined using a Laser Diffraction Particle Size Analyser (Beckman Coulter LS 230, Coulter Corporation, Miami, USA). A wet module was used, where ethanol was used as a carrier and MilliQ-water was used for the background solution. The measurement of each sample was performed in duplicate and the results were reported as averages and were expressed in volume units (Publications 1–4).

4.3 Preliminary study for extrusion

A preliminary study with a full factorial experimental design (Publication 1) was performed in order to understand the effects of extrusion processing conditions on bran modification and the macrostructure of rye bran extrudates. Three different particle sizes of rye bran (coarse: 440, medium: 143 and fine: 28 μm) were used to produce 36 different extrudate samples using a co-rotating twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Dreieich, Germany). The die exit of the extruder was 5 mm in diameter. For the solid feed, a volumetric co-rotating twin-screw feeder (Brabender, Duisburg, Germany) was used. A constant feed rate of 67 g/min and a screw speed of 300 and 500 rpm was used in the experiments. Two different temperature profiles, i.e. low: 40-70-75-75-90-110-110 °C and high: 40-70-75-90-95-130-130 °C, were used in sections 1–6 and in the die, respectively. Two different feed moistures were used at either 17% or 19%. Two different water addition regimens were used for the 17% moisture feed: an in barrel–water feed (IB) and preconditioning (PC). A peristaltic pump (Watson Marlow 505S, Wilmington, MA, USA) was used for the IB feed moisture, where the distilled water was fed into the extruder barrel though the injection gate for liquids (in section 1). Whereas in the PC regimens, the moisture content of the bran was adjusted prior to extrusion and was mixed using a spiral mixer (Diosna SP 12 F/E, Dierks & Söhne, Osnabrück, Germany). The required amount of water was added gradually with continuous high-speed mixing, during which moisture loss was prevented by using a cover. Mixing was continued for another 10 minutes after completion of the water addition. Preconditioned bran was then hydrated overnight at 4 °C. For the 19% feed moisture (only IB), the water was fed into the extruder barrel immediately after feeding the dry materials. Extruded samples of each extrusion experiment were collected and dried in an oven at 105 °C for 20 minutes and cooled to room temperature.

Based on the preliminary trials, it was determined that the screw speed and particle size of the feed material were the most important processing parameters. The extrudates with the highest expansion and lowest piece density were achieved with a combination of a high screw speed and small bran particle size. Neither the barrel temperature profile nor the hydration regimen influenced the expansion properties. However, increasing the feed moisture from 17% to 19% slightly reduced the expansion rate. Severe extrusion processing increased the SDF content of the extrudates. On the other hand, preconditioning reduced the IDF content. Other processing parameters did not influence the DF components of extruded rye bran.

Based on the results of the preliminary study, final experiments were designed with four extruded samples of each particle size. Both 300 and 500 rpm screw speeds were studied in Publication 1 since the macrostructural properties were significantly influenced by screw speed. As the hydration regimens were the only parameters

influencing the DF contents, both IB and PC were chosen (Publications 1−2). In all experiments of Publication 1, a low feed moisture content (17%) and high temperature profile: 40-70-75-90-95-130-130 °C were used. Since high screw speed and low feed moisture improved the expansion, a high screw speed (500 rpm) and a low feed moisture (17%) were chosen in all experiments of Publication 2. To avoid burning issue a low temperature profile: 40-70-75-75-90-110-110 °C was used (Publication 2).

4.4 Extrusion processing

A full factorial experimental design was used for extrusion (Publications 1−2) with a twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Dreieich, Germany). A constant feed rate (67 g/min) was maintained for all experiments. Two different screw speed were used: 300 (Publication 1) and 500 (Publications 1−2) rpm. The temperature profile used was: 40, 70, 75, 90, 95, 130 and 130 °C (Publication 1) and 40-70-75-75-90-110-110 °C (Publication 2) in sections 1–6 and in the die, respectively. The feed moisture used was 17% with two water addition regimens: IB and PC. The rye bran was thoroughly mixed using a high-speed spiral mixer (Diosna SP 12 F/E, Dierks & Söhne, Osnabrück, Germany). Extruded samples of each extrusion experiment were collected and dried immediately in an oven for 20 min at 105 °C, after which they were cooled to room temperature. During extrusion, the torque, die pressure, and die temperature were recorded, and the SME was calculated. The experimental design for rye bran extrusion (Publication 1) and product optimization (Publication 2) are shown in Table 2.

A twin-screw extruder (APV MPF 19/25, Baker Perkins Group Ltd., Peterborough, UK) was used in Publications 3−4 with screw speeds ranging between 250-350 rpm at a constant feed rate of 60 g/min. The temperature profile from the feed inlet to the die exit was: 80, 95, 110, 120 °C (sections 1 to 4, respectively). Water was pumped into the extruder barrel to obtain the desired moisture content in the extrudates. A pair of co-rotating twin screws and a 3 mm die were used in all the extrusion experiments. For feeding, a co-rotating twin screw feeder (K-Tron Soder, Niederlenz, Switzerland) was used. Extruded products were collected from the die exit and dried immediately in an oven for 30 and 60 min for puffs and flakes, respectively at 100 °C.

The torque values were monitored during the extrusion (Publications 1−4) and the specific mechanical energy (SME) was calculated according to the protocol published by Hu et al., 1993; with Eq. 1:

$$SME\ (\text{kW h kg}^{-1}) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (1)$$

Where ω is the screw speed (rpm), ω_r is the maximum screw speed of the extruder used (Pub. 1–2: 1100 rpm; Pub. 3–4: 500 rpm), τ is the torque (%), Z_r is the maximum

power capacity of the extruder (Pub. 1–2: 16 kW; Pub. 3–4: 2 kW) and Q is the feed rate (kg/h). The experimental design for the rye bran extrusion (Publications 1–4) is shown in Table 2.

Table 2. *Experimental design for rye bran extrusion and product optimization with varying particle sizes and extrusion process parameters in Publication 1–4.*

Publication	Sample code	Form	Rye bran (%)	Bran category & size (µm)	Hydration regimen	Screw speed (rpm)
Pub. 1	IB 300	Puff	100	Coarse (440)	IB	300
	IB 500	Puff	100	Coarse (440)	IB	500
	PC 300	Puff	100	Coarse (440)	PC	300
	PC 500	Puff	100	Coarse (440)	PC	500
	IB 300	Puff	100	Medium (143)	IB	300
	IB 500	Puff	100	Medium (143)	IB	500
	PC 300	Puff	100	Medium (143)	PC	300
	PC 500	Puff	100	Medium (143)	PC	500
	IB 300	Puff	100	Fine (28)	IB	300
	IB 500	Puff	100	Fine (28)	IB	500
	PC 300	Puff	100	Fine (28)	PC	300
	PC 500	Puff	100	Fine (28)	PC	500
Pub. 2	IB 15	Puff	15	Coarse (440)	IB	500
	IB 30	Puff	30	Coarse (440)	IB	500
	PC 15	Puff	15	Coarse (440)	PC	500
	PC 30	Puff	30	Coarse (440)	PC	500
	IB 15	Puff	15	Fine (28)	IB	500
	IB 30	Puff	30	Fine (28)	IB	500
	PC 15	Puff	15	Fine (28)	PC	500
	PC 30	Puff	30	Fine (28)	PC	500
Pub. 3	RB-0	Puff	0	n/a	IB	345
	RB-10	Puff	10	Fine (24)	IB	345
	RB-0	Flake	0	n/a	IB	250
	RB-10	Flake	10	Fine (24)	IB	260
Pub. 4	RB-0	Puff	0	n/a	IB	350
	RB-40	Puff	40	Coarse (361)	IB	350
	FB-40	Puff	fermented 40	Coarse (361)	IB	350

The variables which changed in each publication are underlined.

4.5 Properties of extrudates

4.5.1 Chemical analysis

The chemical composition of the raw materials (Publications 1–2) and extruded samples (Publications 1, 3 and 4) were analysed for the total starch, DF content, protein, fat, moisture and ash content. The starch content (Publications 1–4) was analysed using AACC method no. 76.13 (AACC, 1999). The analysis of the TDF content (Publications 1–4) was carried out according to AOAC method no. 985.29 (AOAC, 1990) and the IDF and SDF content (Publications 1–4) were analysed using AOAC method no. 991.43 (AOAC, 1992). The total protein content (Publications 1, 2 and 4) was analysed according to AACC method no. 46–11A (AACC, 2003) and the fat (Publications 1, 2 and 4) contents were analysed using AOAC method no. 922.06 (AOAC, 2000). The moisture content (Publications 1–4) was analysed by drying the samples in an oven at 105 °C for 3 hours and the ash content was gravimetrically analysed by burning at 550 °C in a muffle furnace. The total starch and fibre analyses were performed as triplicates, whereas the fat and ash were analysed as duplicates. Microbiological, pH and TTA analyses (Publication 4, pp 1959–1960) were carried out for the bran material at the beginning and the end of fermentation according to the protocol published by Kajala et al. (2016). Fermented bran was analysed for the dextran content as described by Katina et al. (2009).

4.5.2 Macrostructure

Extruded samples were analysed for macrostructural properties such as expansion, specific length, and piece density. A total of 20 (Publications 1–3) and 30 (Publication 4) replicates were used to characterize the macrostructural properties. A Vernier calliper was used to obtain the length and the diameter was measured at three different points of an extrudate ribbon. The average diameter value represented the diameter of each sample.

The expansion rate was calculated with Eq. 2:

$$Expansion\ rate\ (\%) = \frac{D_e}{D_d} \times 100\% \quad (2)$$

Where D_e is the average diameter of the extrudate sample (mm) and D_d is the diameter of the die (5 and 3 mm).

The specific length was calculated with Eq. 3:

$$Specific\ length\ (\mathrm{m.kg^{-1}}) = \frac{L_e}{m_e} \quad (3)$$

Where L_e is the length of the extrudate sample (m) and m_e is the mass of the sample (kg).

The piece density was calculated with Eq. 4:

$$Piece\ density\ (\mathrm{kg.m^{-3}}) = \frac{4 \times m_e}{\pi \times (D_e)^2 \times L_e} \quad (4)$$

Where m_e is the mass of the sample (kg), D_e is the average diameter of the extrudate sample (m) and L_e is the length of the extrudate sample (m).

4.5.3 Microstructure

Light microscopy

Pieces of rye bran and extrudates were embedded according to the instructions provided for the Leica Historesin embedding kit (Heidelberg, Germany). 2 µm sections of each sample were prepared using a Rotary Microtome HM 355S (Microm Laborgeräte GmbH, Walldorf, Germany) and a tungsten carbon knife. Protein was stained with aqueous 0.1% (w/v) acid fuchsin (BDH Chemicals Ltd., Poole, Dorset UK) in 1.0% acetic acid for 1 min, and β-glucan was stained with aqueous 0.01% (w/v) calcofluor white (Fluorescent Brightener 28, Aldrich, Germany) for 1 min. In exciting light (excitation, 400–410 nm; emission, >455 nm), intact cell walls stained with calcofluor appeared blue and proteins stained with acid fuchsin appeared red. Starch was unstained and appeared black. The samples were examined with an Olympus BX-50 microscope (Olympus Corp., Tokyo, Japan). Micrographs were obtained using a PCO SensiCam CCD colour camera (PCO AG, Kelheim, Germany) and the Cell^P imaging software (Olympus).

X-ray microtomography

Puffed extruded samples (Publications 1–3) were prepared by cutting an extruded ribbon into 10 mm pieces (axial section) with an electric saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan). Flake samples (Publication 3) were analysed as is without further preparation. The X-ray microtomography (XMT) was performed using a desk top XMT system (Model 1172, SkyScan, Aartselaar, Belgium) consisting of an X-ray tube, an X-ray detector, and a CCD camera. A modified method (Sozer et al., 2011a, b) was used for the XMT analysis in which the X-ray tube was operated at a voltage of 40 kV and a current of 250 µA to obtain the optimum contrast between the void (air cells) and matter (cell walls). X-ray data was collected using a 12-bit cooled CCD camera (512 x 1024 pixels). Samples were rotated by 180° during 18 min of scanning with a voxel size of 11.65 µm × 11.65 µm × 11.65 µm. The raw images or initial X-ray radiographs were taken at every 0.7° rotation. Analysis of the scanned radiographs was performed using the NRecon reconstruction software (v.

1.6.6). A 3-D image was created when the stack images were combined. Later from the 3-D image, 2-D cross sectional images were taken. In order to reduce the number of artefacts, beam hardening correction was set to 40%. In the 2-D XMT images, the air cells appeared black, whereas the cell walls of the solid matrix appeared grey. When the reconstructed 2-D slices were ready, they were loaded into the CTAn software package (v. 1.12, Skyscan, Belgium) to obtain the porosity, cell wall thickness (t) and cell diameter (D) parameters. The analyses were performed in triplicate (Publications 1–2) and quintuplicate (Publication 3). The results were then reported as mean value of 3 or 5 replicates ± SD.

4.5.4 Texture

A uniaxial compression was performed using a TA-HDi (HD3071, Stable Micro Systems Ltd., United Kingdom) texture analyser or a TA.XT2 (Stable Micro Systems Ltd., Godalming, United Kingdom) to determine the mechanical properties of the extrudates. A heavy-duty TA-HDi was used for hard samples (Publications 1 and 3), whereas comparatively less hard and crispy samples (publication 2 and 4) were analysed using a TA.XT2. The TA-HDi and TA.XT2 texture analysers were equipped with 250 and 30 kg load cells, respectively and with a cylindrical aluminium probe of 36 mm (Publications 1–2) and 25 mm (Publications 3–4) in diameter. Texture analysis samples were prepared by cutting the extruded ribbons into 10 mm pieces (radially) with an electric saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan) with (Publications 1–2) or without (Publications 3–4) equilibrated at a relative humidity of 43% at 21 °C. Extruded samples were deformed at 70% strain and the test speed of the probe was 1 mm/s. The acquisition rate remained constant for all 200 (puffs) points/s experiments. Each measurement was performed for 20 (Publications 1–2), 30 (Publication 3) and 80 (Publication 4) replicates.

Due to the structural differences, the protocol used for puffed samples was not suitable for flakes (Publication 3) and therefore a thick bed of flakes was used instead of an axial section. A TA-HDi Texture Analyser (HD3071, Stable Micro Systems, United Kingdom) equipped with a 250 kg load cell and a 5-blaled Kramer shear cell ($90 \times 66 \times 82$ mm^3) was used to apply the mechanical stress to the bed of flakes. The samples were deformed at 25% strain with an acquisition rate of 400 points/s with a cross head speed of 1 mm/s. The samples were analysed as is and about 35 g were weighed to form 20 mm thick bed in the Kramer shear cell. A total of 10 replicates were made for each flake sample. The test distance used was set to 25 mm to ensure the blades passed through the respective slots of Kramer shear cell by 3 mm.

Force–displacement (f–d) curves were obtained during the analysis in order to assess the mechanical properties of the extruded samples using Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK). In the f–d curve, the number of peaks represented the number of cell wall ruptures during uniaxial compression and F_{max} was

the maximum force needed to initiate cell wall crack, which is commonly known as the hardness of the samples. Different approaches were used to describe the hardness (F_{max} and crushing force) and crispiness (crispiness work, cw and crispness index, ci) and this was calculated using the following equations. High C_i and low C_w represented crispy samples, whereas low C_i and high C_w exhibited less crispy samples.

The crushing force was calculated with Eq. 5:

$$Crushing\ force\ (N) = \frac{A}{l} \tag{5}$$

Where *A* is the area under the f-d curve (N mm) and *l* is the distance of compression (mm).

The crispiness work (C_w) was calculated with Eq. 6 (Van Hecke et al., 1998).

$$C_w\ (N\ mm) = \frac{A}{N} \tag{6}$$

Where *A* is the area under the f-d curve (N mm) and *N* is the number of peaks.

The crispiness index (C_i) was calculated with Eq. 7 (Heidenreich et al., 2004).

$$C_i = \frac{L_N}{A \times F_{mean}} \tag{7}$$

Where L_N is the normalized curve length (length of actual curve/F_{max}), *A* is the area under the f-d curve (N mm) and F_{mean} is the sum of the actual force values in the data file divided by the number of data points (N).

4.5.5 Viscosity

The extruded products (Publication 4) were ground using a Retsch mill at a speed of 6000 rpm using a 1 mm sieve. A Rapid Visco Analyzer (RVA-Super4, Newport Scientific, Warriewood, Australia) was used to analyse the viscosity of the ground extruded samples. For each experiment, ground samples of about 5 g were dispersed into 25 ml of distilled water to achieve a 14% moisture basis in order to obtain a homogeneous and viscous slurry. A pre-installed calculator using Thermocline software (TCW3) was used to calculate the number of samples and water prior to the experiment. An analytical balance was used to weigh the samples and water at 0.001 g accuracy. At first, water was weighed carefully in an empty canister and then weighed sample was slowly dispersed in the water. The experiment time was set for 180 min with an initial stirring speed 960 rpm for 10 sec followed by a constant 120 rpm speed. The experiment temperature was kept constant (37 °C) throughout the

experiment. At the end of the experiment, the slurry was cooled to 25 °C and the final viscosity was obtained using the Thermocline software. The results were reported by the mean value of three replicate ± SD.

4.5.6 Sensory perception

A trained sensory panel (VTT Technical Research Centre of Finland) profiled the sensory attributes of the extrudates in duplicate sessions. All members (n = 12) of the sensory panel have been trained and have passed the basic taste, odour, and colour vision test. The panellists familiarized themselves with the products in a training session, in which the key attributes relevant to the product category were defined. The sensory profiling method was a descriptive analysis (Lawless & Heymann, 2010) and the defined sensory attributes were: hardness, crispiness, coarseness, thickness, sliminess and intensity of the overall flavour. Reference samples were used for most attributes to define the extremes and all descriptors were verbally anchored. All the studied sensory attributes were evaluated using a 10 cm line scale ranging from ‘‘not at all = 0’’ to “extremely = 10’’. The lighting was adjusted to hide the colour of the products during the evaluation sessions. Samples were offered as 2 cm pieces and the panellists were asked to place the sample vertically between their molars when evaluating the crispiness and hardness. The panellists were instructed (e.g. “chew the sample using your back teeth until the sample is ready to be swallowed and assess the properties”) on how to treat the samples during the evaluation (Table 1, Publication 4). The samples were blind-coded with 3-digit numbers and were served in a randomized order. Water was served to clean the palate between different samples. The scores were collected and recorded during the session using the Compusense Five, Ver 5.4.15, CSA Computerized Sensory Analysis System software (Compusense Inc., Guelph, ON, Canada).

4.5.7 Starch hydrolysis index

Determination of the starch hydrolysis index (HI) of the extruded products was performed using a protocol published by Sozer et al. (2014). Extruded samples were ground using a Retsch mill (Retsch Ultra Centrifuze Mill ZM 200) at a speed of 6000 rpm using a 1 mm sieve. In a beaker, ground extruded samples of about 1.4–2.0 g (the weight was adjusted to ensure 1 g starch in the sample) were taken and the same amount of distilled water (1:1) was added to the sample. A 0.05 mol l^{-1} sodium potassium phosphate buffer ($KH_2PO_4 \cdot Na_2HPO_4 \cdot 2H_2O$, pH = 6.9) was prepared and 100 ml of the buffer was added to each beaker. The pH of the solution was then adjusted to 6.9 using either 0.1 mol l^{-1} NaOH or HCl. A porcine pancreatic α-amylase (110 U, 1 ml per 1 g starch, Sigma-Aldrich Co. LLC, USA) was added and the solution was incubated for 180 min at 37 °C. Samples were taken at every 30 min with a pipette for maltose analysis. The Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA,

USA) software was used to calculate the area under the curve (AUC). Incremental AUC was calculated using a formula [e.g. 0 min = ½ × (abs_0 + abs_{30}) × (t_{30} − t_0)] for the trapezoidal area for 0, 30, 60, 120 and 180 min (here, abs = mg of maltose per 1 g soluble starch). Ground white wheat bread (air dried) was used as a reference product. A starch hydrolysis test of wheat bread was repeated with each batch of extruded samples. The hydrolysis indices of the extruded samples were calculated by comparing the AUC to that of wheat bread (average of three analyses). The formula used for the calculation was: [HI = (AUC of extruded products / Average AUC of white wheat bread) × 100]. The analysis of the extruded samples was performed in triplicates and the HI values were calculated for each time interval. The HI final results were reported as mean ± SD value of three replicates.

4.6 Mastication trial

4.6.1 Participants

Participants for mastication trials were enrolled through group emails, public advertisements and through bulletin boards near the study location. Only young healthy females were recruited to avoid possible and unnecessary variation in mastication patterns due to gender effects. The eligibility of the participants was checked through a screening questionnaire. The eligibility criteria included women aged between 20 and 40 years, a body mass index (BMI) between 18.5 and 25 $kg.m^{-2}$ (Table 3) and a stable body weight (±4 kg during the previous year) with the habit of eating breakfast. All participants were of normal body weight, they had no abnormal eating behaviour (according to the Eating Disorder Diagnostic Scale; Stice et al., 2000), no missing teeth (except 3rd molars) and no diagnosed acute temporomandibular disorders (self-reported). Moreover, pregnant, or lactating women, persons with dietary restrictions (e.g., celiac disease sufferers, those with allergies or aversions to cereal foods or high carbohydrate foods) and smokers were excluded. Females who fulfilled the inclusion criteria and were interested in being enrolled were invited to an information visit to provide their written informed consent for their participation in the study. In total fifteen (Publication 3) and twenty-six (Publication 4) female participants were included in the mastication trials. One movie ticket per study visit was offered to all the participants to compensate for their time and effort. The ethical principles of good research and clinical practice described in the declaration of Helsinki were followed when the study was conducted. Ethical approval was obtained from the "Research Ethics Committee of the Hospital District of Northern Savo, Finland" and "The Coordinating Research Ethics Committee of the Helsinki and Uusimaa Hospital District" for Publications 3 and 4, respectively.

4.6.2 Procedure

Electromyography (EMG) measurements of extruded products through mastication trials were performed according to Pentikäinen et al. (2017). A crossover and single-blind design were followed for all the EMG and mastication trials. The experiment time was scheduled at 8–11 am in the morning and the participants were instructed to have breakfast 1–1.5 hours before the study visit. Before the actual mastication trials, all participants went through the study protocol with bread samples to familiarize themselves with the study procedure. All the participants attended one study visit during which all the extruded samples were served in a randomized order to be masticated in three replicates. The samples were blind coded using 3-digit numbers and each product was served in three separate portions and masticated in direct succession. Each puffed portion consisted of two pieces and flake portions were served in 1 tablespoon helpings (Table 3). The participants were instructed to continue to masticate each portion until a bolus was ready for swallowing. At that point they expectorated the bolus into a plastic cup instead of swallowing it. The cup containing the bolus was collected and kept on ice. There was a 2 min break between different extruded products during which the mouth was rinsed with water. When all the study products had been masticated the participants were offered three pieces of wheat bread (Publication 3) and chewing gum (Publication 4) and were asked to chew each for 20 seconds. Individual oral processing data for the wheat bread or chewing gum were used as references for force parameters to get an indication of the relative force required to masticate each of the study products. In order to support the data analysis, all the mastication sessions of each participant were video recorded.

Table 3. *Mastication trials, study products and participants.*

	Publication 3	**Publication 4**
Study products	Puffs and flakes with or without 10% bran (RB-0 and RB-10)	Puffs with or without 40% bran and with 40% fermented bran (RB-0, RB-40 and FB-40)
Portion size, g (length × width)	Puffs: 2 pieces: 1.85 g (3.5 × 1.6–1.7 cm) Flakes: 1 spoon: 10.5 g (2.2–2.3 × 0.26–0.32 cm)	2 pieces: 1 g (2 × 1–1.8 cm)
Number of participants	15	26
Age (year)	Range: 20–40 Av: 24.6 ± 4.4	Range: 20–40 Av: 31.7 ± 7.5
BMI ($kg.m^{-2}$, mean ± SD)	22.0 ± 1.4	22.2 ± 1.9

4.6.3 Electromyography measurements

The electrical activity of the masticatory muscles (i.e. the masseter and temporal muscles) during mastication was measured using electromyography (EMG) to characterise the mastication process (Publications 3–4). A NeurOne system (Mega Electronics, Kuopio, Finland) with disposable dermal Ag/AgCl electrodes was used

for the EMG measurements. The masticatory muscles were identified by touching the face when the participants gritted their teeth. Bipolar electrodes were placed on the masseter and temporal muscles on both sides of the face after cleaning the face with 70% ethanol. On the cervical vertebra, a reference electrode was placed. Throughout the mastication session, the EMG activity was measured and recorded continuously. The data blocks for each mastication period were isolated in the EMG acquisition system using markers added to the EMG data. A wavelet filter was applied to the EMG time series to extract the onset, duration, and amplitude of each mastication event. A chemometric technique was applied on the extracted data followed by squaring and smoothing. High frequencies and background fluctuations were eliminated from the EMG time series (Pentikäinen et al., 2014). From the derivative curve, individual chews were recognised. Normalization was carried out of the chewing force and work parameters using mastication data obtained from the bread or chewing gum trials.

To analyse the EMG data, Matlab® (The MathWorks Inc., Natick, MA, USA) software was used, and all the mastication parameters were computed separately for all four muscles monitored. The calculated mastication properties for each extruded product were: the number of chews, the chewing time, the force/chew, and the total work (EMG activity time × force/chew). The relative chewing force and relative work parameters were calculated in relation to the chewing process of wheat bread (Publication 3) and chewing gum (Publication 4), e.g., the relative chewing force was calculated from the highest EMG amplitude of the products normalized to the highest EMG amplitude for wheat bread or chewing gum, whereas the relative work was calculated by multiplying the EMG activity time by the relative chewing force.

4.7 Bolus properties

4.7.1 Saliva uptake

The saliva uptake was calculated for the bolus samples based on the moisture content of the original extruded products and the moisture content of the bolus samples (Pentikäinen et al., 2014). Fresh and wet bolus samples were collected from fifteen (Publication 3) and twenty-six (Publication 4) individual participants during the mastication trials. Small amounts (0.5 g) of the bolus from each participant were taken in an aluminium moisture cup (in triplicates, without mixing with each other) and were dried overnight in an oven at 105 °C. The bolus samples were weighed again after overnight drying. The saliva uptake of the boluses was obtained by subtracting the moisture content of the extruded products from the moisture content of boluses. The results (average of three replicates) were first obtained for each individual participant and later reported as the mean ± SD of fifteen and twenty-six participants for each extruded product.

4.7.2 Particle size distribution

Particle disintegration of the bolus samples (Publications 3–4) was examined using the protocol published by Pentikäinen et al. (2014). The bolus samples were obtained from eight (Publication 3) and twenty-six (Publication 4) individual participants. The bolus samples were diluted in a beaker with 100 ml of distilled water. A magnetic stirrer was used to stir the solution at a constant speed of 220 rpm for 25 min. After continuous stirring, the beaker containing the diluted bolus was allowed to stand for 5 min in order to allow larger particles settle at the bottom. Turbid liquid containing the smallest particles that could not be imaged was gently removed from the top without agitation. After removing the turbid liquid, another 100 ml of distilled water was added to increase the sample volume. Bolus particles with sufficient liquid were poured onto petri dishes for imaging. Digital images were taken for each petri dish with a camera fitted with a stand at the same height and with the same camera settings. The Cell^P imaging software (Olympus, Germany) was used to determine the particle areas and later visualized using a set of granulometric curves.

4.7.3 Viscosity of the boluses

The viscosity of the bolus samples (Publication 4) was analysed according to the method described in "viscosity of the extrudates" section. A substantial amount of the bolus samples was required for the viscosity analysis, thus the boluses were collected from a single person (who was not one of the 26 mastication trial participants). For the analysis, about 8 g sample of bolus was required for each experiment, which was obtained from the same person. The sample was added to 25 ml of distilled water to reach desired concentration. The final viscosity of the boluses was obtained after 180 min and was reported as the mean ± SD values of three replicates.

4.8 Statistical analyses

The overall differences between the study products were assessed using a one-way analysis of variance (ANOVA). For a pairwise comparison (Publications 1–4), the least significant difference (LSD) and Tukey's honest significant difference (HSD) were used in a post-hoc analysis with a significance level of 0.05. Linear correlations between different variables were calculated using a two-tailed Pearson bivariate correlation at significance levels of 0.01 and 0.05 (Publications 1–2). Statistical analyses were conducted using the SPSS Statistics 19-24 software (SPSS Inc., Chicago, USA).

5 Results

5.1 Compositional and structural characterization of extrudates

5.1.1 Impact of milling, fermentation and extrusion processing on dietary fibre composition (Publication I)

The processing of bran either by milling or fermentation resulted in variations in the DF composition of rye bran which has been further altered by extrusion. Particle size reduction from 440 to 28 µm reduced both the IDF and TDF but increased the SDF contents (Table 4, Publication 1). Medium (143 µm) and coarse (440 µm) rye bran differed only in the IDF content. Medium rye bran had 8% lower IDF compared to coarse rye bran. In contrast, fine rye bran (28 µm) had 13–19% lower IDF but had 10–15% higher SDF contents than medium and coarse rye bran (Figure 3). As a result, fine rye bran had 7–13% lower TDF contents compared to medium and coarse rye bran.

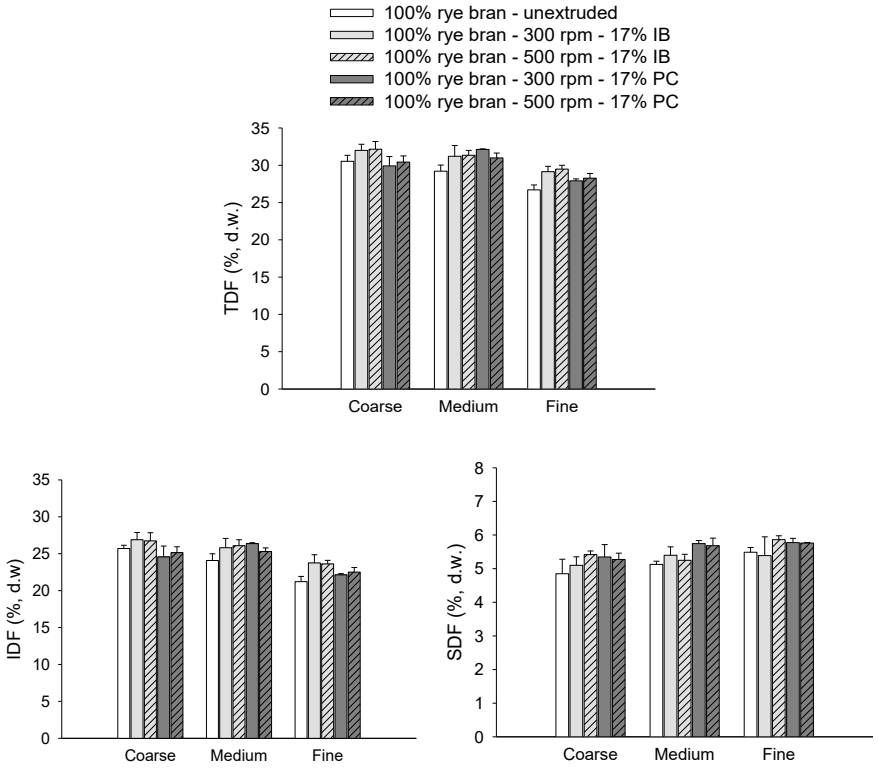

Figure 3. *TDF, IDF, SDF contents of unextruded and extruded coarse, medium and fine particle sized rye bran.*

However, an increase in both the IDF (increased by 7−12 %) and TDF (increased by 6−11 %) content was observed for both medium and fine bran after extrusion (Table 4, Publication 1) regardless of the screw speed and different hydration regimens (IB and PC). In general, fine rye bran extrudates had the highest SDF but lowest amounts of IDF and TDF (Figure 3). Preconditioning increased the SDF contents only in medium rye bran extrudates regardless of the screw speed (Figure 3). However, parameters such as the screw speed and feed moisture had no significant effect on the DF content of the rye bran extrudates.

High fibre puffs and flakes were prepared by incorporating 10% fine rye bran into endosperm rye flour (RB-10). Rye bran supplementation led to a significant increase in the IDF (67% in puffs and 50% in flakes) and SDF (15% in puffs and 20% in flakes) content both for puffs and flakes compared to 100% endosperm rye flour (RB-0) extrudates. Despite the low bran addition level compared to other studies reported in this thesis, the TDF contents of RB-10 puffs and flakes varied between 8−9% also enabling a high fibre claim (Table 2, Publication 3).

The incorporation of 40% coarse (361 µm) native (RB-40) and fermented (FB-40) rye bran in rye-flour-based extrudates significantly increased the IDF and SDF content of 100% endosperm rye flour (RB-0) extrudate. The IDF content was increased by 256% in RB-40 and 211% in FB-40, whereas the SDF was increased by 71% in RB-40 and 129% in FB-40 compared to RB-0 extrudates (Table 3, Publication 4). In FB-40, the IDF content was 13% lower than RB-40, whereas the SDF content was 25% higher. However, the TDF contents of both RB-40 and FB-40 extrudate came to 22%, which was approximately 3-fold higher than that of the RB-0 (8%) extrudate.

5.1.2 Rye bran induced macro- and micro-structural changes (Publications I–IV)

Macro-structural changes (Publications I–III)

The use of fine rye bran caused a notable increase in the expansion rates of the extrudates, particularly for those processed at high screw speeds (Figure 4). Preconditioning of the bran-flour mix led to better expansion even at low screw speeds (Figure 4). However, for medium rye bran extrudates regardless of different hydration regimens a low screw speed gave the lowest expansion rate. In general, a higher piece density was observed when rye bran was extruded as such. However, the piece density was reduced with a particle size reduction.

Rye bran when mixed with endosperm rye flour led to a better macrostructure compared to extrusion of rye bran as such (Publication 2). A particle size reduction from 440 to 28 µm increased expansion, reduced the piece density and specific length of rye bran supplemented extrudates. An increase in the bran concentration from 15 to 30% significantly ($p < 0.05$) reduced the expansion and increased the piece density.

The expansion rate of the extrudates was positively influenced by pre-conditioning of the bran-flour mix particularly for fine rye bran at a 30% addition level, while in-barrel water fed extrudates were less expanded.

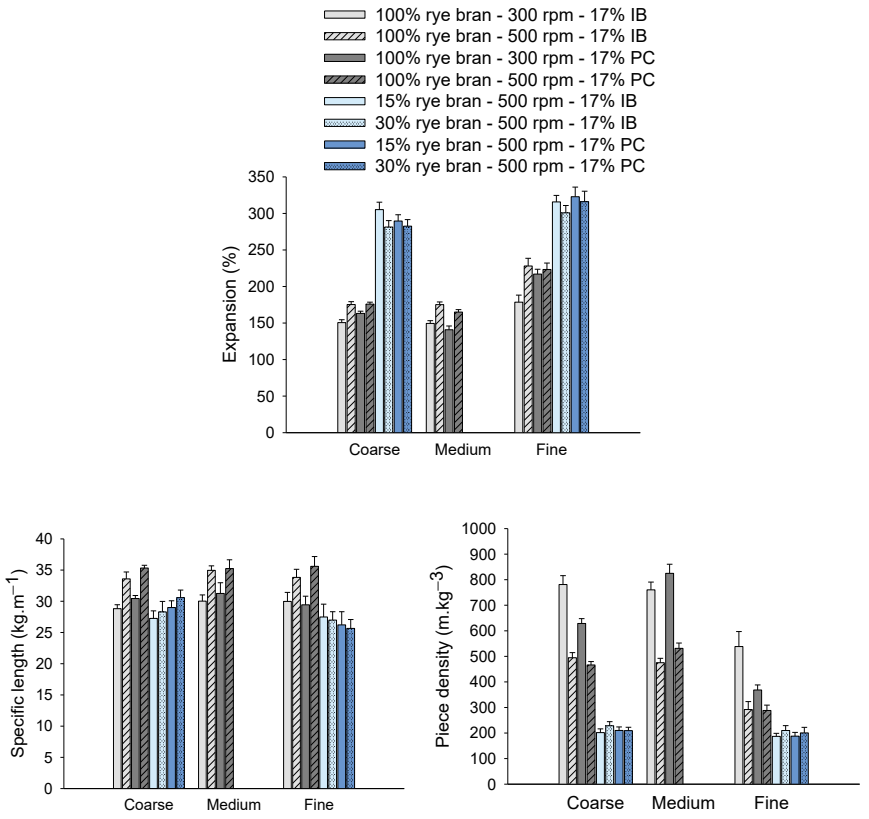

Figure 4. *Macrostructural properties of the extrudates made of 100% (Publication 1), 15 and 30% rye bran (Publication 2).*

A significant negative correlation ($r = -0.81$, $p < 0.05$) between the expansion rate and particle size indicated that lowering the bran particle size increased the expansion rate (Publication 2). The specific length of the extrudates was positively correlated ($r = 0.74$, $p < 0.05$) with the particle size and negatively ($r = -0.83$, $p < 0.05$) correlated with the expansion rate, i.e. lowering the particle size of the bran lowered the specific length and increased the expansion in the radial direction. The expansion rate and piece density were negatively correlated (Publication 2) with each other ($r = -0.88$, $p < 0.01$). Increasing the screw speed increased the specific length and reduced the piece density. Neither a particle size reduction nor changes in the hydration regimen had any impact on the specific length.

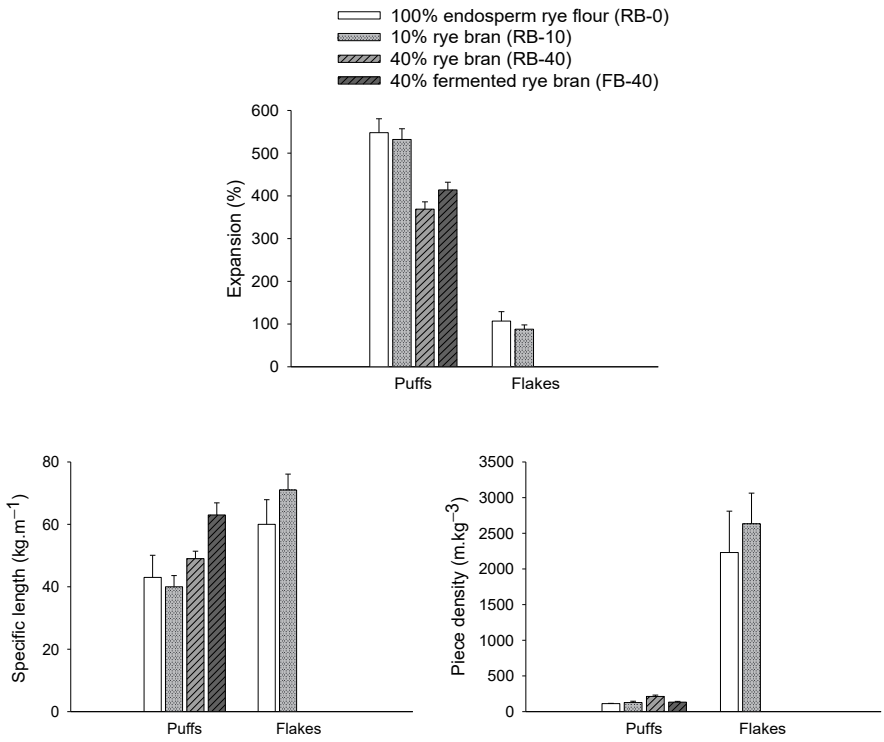

Figure 5. *Macrostructural properties of rye bran supplemented puffs and flakes with varying structural characteristics.*

The incorporation of 10% rye bran caused a lower expansion rate and a higher specific length and piece density in both puffs and flakes (Publication 3). In general, the flakes had a denser structure than the puffs (Figure 5).

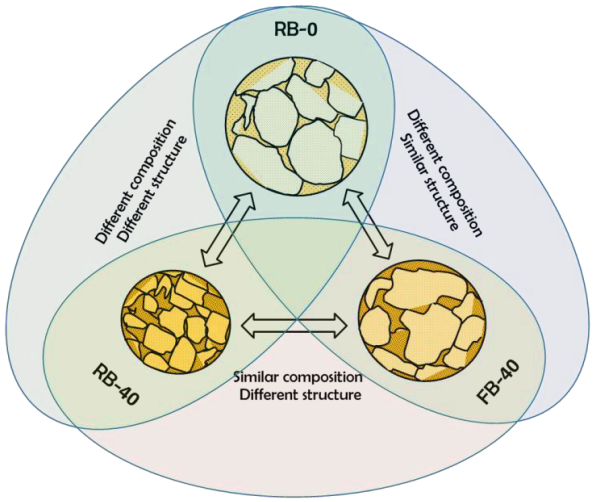

Figure 6. *A schematic overview showing the structural differences between the extrudates made of 100% rye flour (RB-0), 40% native rye bran (RB-40) and 40% fermented rye bran (FB-40).*

Extrudates made with 100% endosperm rye flour (RB-0) had the highest expansion, whereas the incorporation of 40% rye bran resulted in less expanded and denser structures (Publication 4). Fermentation of rye bran (FB-40) notably increased the degree of expansion and specific length compared to the extrudates made of unfermented (RB-40) rye bran (Figure 5 and 6).

Micro-structural changes (Publications I–III)

Milling and extrusion induced microstructural changes were studied using light microscopy. The microstructure of rye bran before and after extrusion processing is shown in Figure 7. In coarse rye bran the structure of the pericarp, testa and aleurone layer remained almost intact and were fully visible, whereas they were broken into smaller pieces in medium rye bran. Further milling into a fine particle size, caused drastic changes in the pericarp, testa and aleurone layers, where almost all the layers were degraded. Larger pieces of the pericarp and testa with only a few unbroken aleurone cells can be seen in fine rye bran.

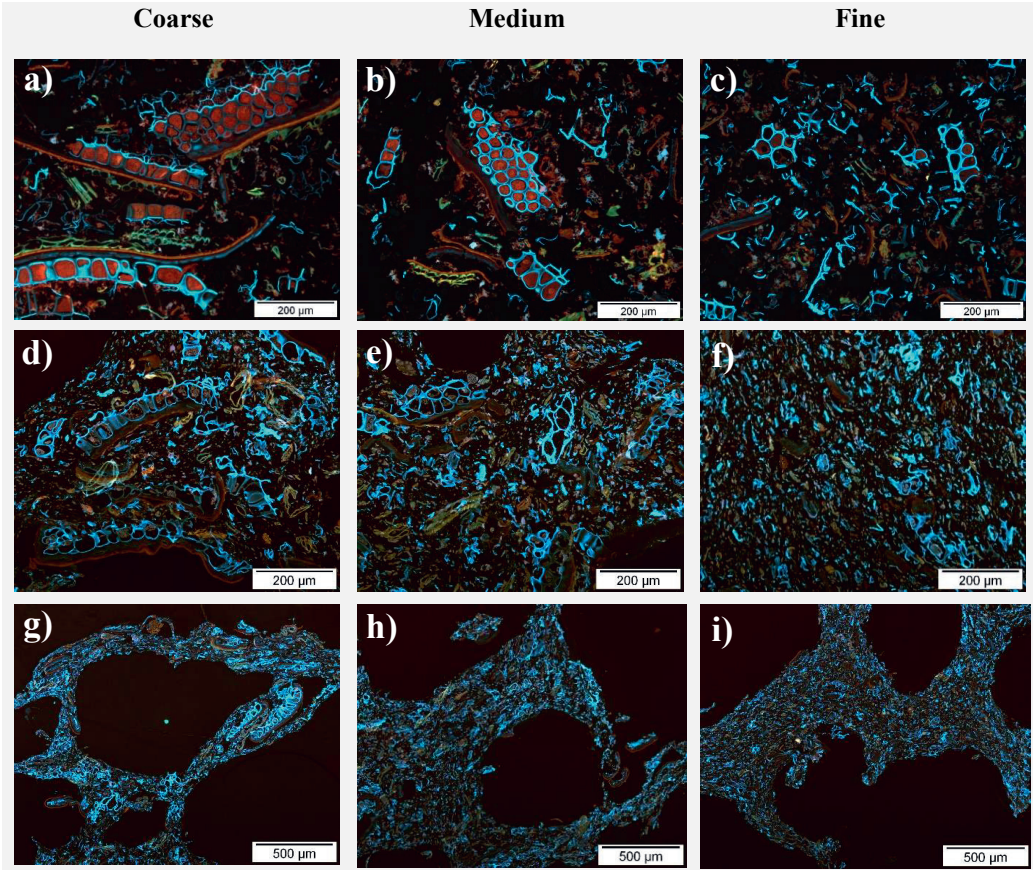

Figure 7. *Light microscopy images of unextruded rye bran are shown in the top row: (a, b and c). The microstructure of extruded rye bran samples at two different magnifications showing the effect of extrusion on bran particles (middle row: d, e, and f) and their alignments (bottom row: g, h and i) within air cell walls. Extrusion processing was performed at a screw speed of 500 rpm with an IB hydration regimen at 130 °C.*

Extrusion processing further degraded the bran structures, while coarse rye bran extrudates still had some large structures, which can be distinguished, whereas there were only few undegraded pieces left in the medium and especially in fine rye bran extrudates (Figure 7).

Protein inside aleurone cells appeared as either bright red or brown in the images of the unextruded samples, whereas after extrusion protein appeared grey (Figure 7). Protein denaturation during extrusion affected its staining properties which caused differences in the appearance of protein before and after extrusion. The high shear forces which occur during extrusion forced the bran particles to align in the direction of the mass flow within the extruder barrel. The bran particles positioned themselves within cell walls of each air bubble.

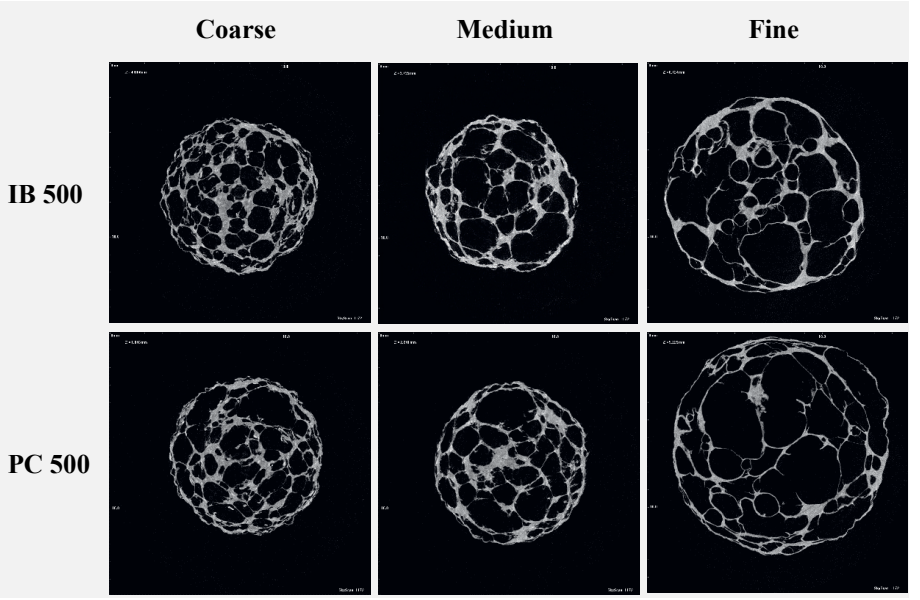

Figure 8. *2-D cross-section images of extrudates made of coarse-, medium- and fine-particle sized rye bran.*

The use of fine rye bran (Publication 1) increased the air cell diameter. Large and spherical air cells were located in the centre of the extrudates with cell anisotropy near the outer surface (Figure 8). The XMT data analysis revealed that a particle size reduction of the rye bran from 440 µm to 28 µm increased the porosity by 31% (Table 6, Publication 1). Fine bran extrudate had the highest average cell diameter (Figure 8) compared to medium and coarse rye bran extrudates. There was no microstructural difference between the medium and coarse bran extrudates (Figure 8). Coarse and medium rye bran extrudates had a large number of small air cells (≈2800−4000) compared to the fine bran extrudates (≈1000).

The effect of particle size was less profound on the average cell wall thickness than the average cell diameter (D). The particle size reduction of bran resulted in air cells with higher cell diameters but with thick cell walls (Table 4). The microstructural properties of coarse and medium rye bran extrudates were not influenced by the hydration regimens. Preconditioning reduced the cell wall thickness in fine rye bran extrudates. A decrease (64%) in the average cell wall thickness to cell diameter (t/D) ratio was observed for fine bran extrudates when compared to coarse rye bran extrudates (Table 6, Publication 1). However, the porosity of the fine rye bran extrudates increased by 6% and the t/D ratio decreased almost by 50% with the preconditioning.

Table 4. *Microstructural properties of the coarse, medium, and fine particle sized rye bran extrudates (Publications 1, 2 and 3) based on XMT image analysis.*

Sample code	Form	Bran category	Porosity (%)	Cell diameter (mm)	Cell wall thickness (mm)
Publication 1: **100% RB extrudates processed at 300 and 500 rpm with 17% IB and PC**					
IB 500	Puff	Coarse	64 ± 0.4 ^{a}	0.49 ± 0.02 ^{a}	0.24 ± 0.01 ^{a}
PC 500	Puff	Coarse	65 ± 0.9 ^{a}	0.51 ± 0.04 ^{a}	0.24 ± 0.01 ^{a}
IB 500	Puff	Medium	65 ± 3.3 ^{a}	0.62 ± 0.07 ^{b}	0.29 ± 0.02 ^{b}
PC 500	Puff	Medium	64 ± 1.7 ^{a}	0.63 ± 0.11 ^{b}	0.29 ± 0.01 ^{b}
IB 500	Puff	Fine	79 ± 1.4 ^{b}	1.17 ± 0.03 ^{c}	0.32 ± 0.04 ^{b}
PC 500	Puff	Fine	84 ± 2.3 ^{c}	1.10 ± 0.14 ^{c}	0.20 ± 0.01 ^{c}
Publication 2: **15 and 30% RB extrudates processed at 500 rpm with 17% IB and PC**					
IB 15	Puff	Coarse	90 ± 1.1 ^{mn}	1.4 ± 0.07 ^{mn}	0.14 ± 0.01 ^{m}
IB 30	Puff	Coarse	86 ± 1.1 ^{m}	1.1 ± 0.06 ^{mn}	0.16 ± 0.01 ^{mn}
PC 15	Puff	Coarse	89 ± 2.7 ^{m}	1.3 ± 0.22 ^{mn}	0.14 ± 0.01 ^{m}
PC 30	Puff	Coarse	87 ± 0.6 ^{m}	1.2 ± 0.07 ^{mn}	0.14 ± 0.00 ^{m}
IB 15	Puff	Fine	89 ± 1.5 ^{m}	1.5 ± 0.18 ^{mn}	0.17 ± 0.01 ^{no}
IB 30	Puff	Fine	89 ± 1.2 ^{m}	1.6 ± 0.11 ^{n}	0.19 ± 0.01 ^{o}
PC 15	Puff	Fine	88 ± 2.2 ^{m}	1.0 ± 0.36 ^{m}	0.15 ± 0.01 ^{mn}
PC 30	Puff	Fine	88 ± 2.7 ^{m}	1.3 ± 0.28 ^{mn}	0.16 ± 0.00 ^{mno}
Publication 3: **100% RF (RB-0) and 10% RB (RB-10) puffs and flakes**					
RB-0	Puff	n/a	96.8 ± 0.4 ^{y}	2.43 ± 0.25 ^{y}	0.12 ± 0.01 ^{x}
RB-10	Puff	Fine	96.5 ± 0.6 ^{y}	2.31 ± 0.21 ^{y}	0.12 ± 0.01 ^{x}
RB-0	Flake	n/a	33.7 ± 4.7 ^{x}	0.18 ± 0.03 ^{x}	0.17 ± 0.01 ^{y}
RB 10	Flake	Fine	36.7 ± 4.6 ^{x}	0.17 ± 0.04 ^{x}	0.15 ± 0.01 ^{y}

Values marked with the same letters (a – c; in Publication 1), (m – o; in Publication 2) and (x – z; in Publication 3) in each macrostructural properties (porosity, cell diameter and cell wall thickness) were not significantly different ($p < 0.05$).

Extrudates with 15 and 30% added rye bran had comparatively higher porosity (Table 4) due to their high starch (74−82%) content (Publication 2). The high ratio of starch to bran diminished the effect of the bran particle size reduction on the porosity and air cell diameter (Table 4). However, the cell wall thickness was significantly increased by particle size reduction and the effect was observed only with the IB hydration regimen (Table 2, Publication 2).

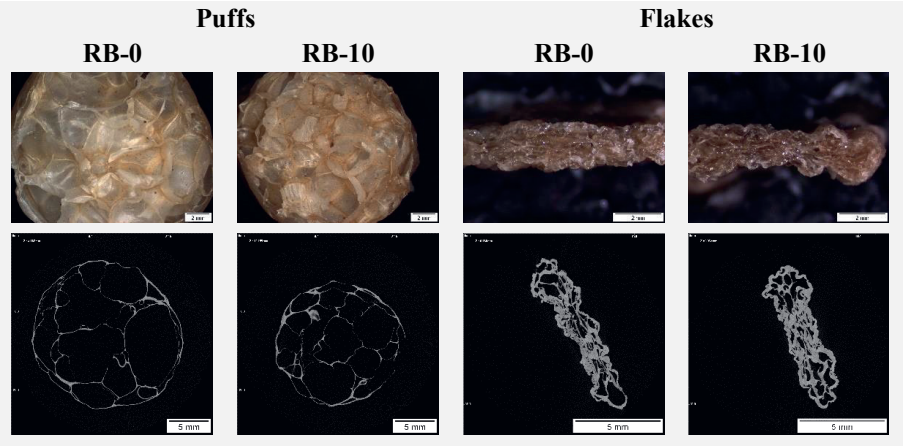

Figure 9. *Stereomicroscopy (the white bar is 2 mm) and 2D-XMT (the white bar is 5 mm) images of extruded puffs and flakes.*

The puffs and flakes had distinct structural features regardless of rye bran addition (Publication 3). The puffs had higher porosity and thinner cell walls compared to the flakes (Table 4 and Figure 9). The cell diameter of the puffs was significantly larger than the flakes with or without 10% rye bran. The microstructural properties of the extrudates were not affected by bran supplementation (10%, 24 μm).

5.1.3 The effect of extrusion and bran modification on the texture and sensory properties (Publications I–IV)

The mechanical properties of the expanded products obtained by instrumental texture analysis are important to help predict the sensory attributes of the products. Crispy products usually consist of a jagged f-d curve representing many simultaneous fracture events. The maximum force required to cause a cell wall rupture during a compression test represents the hardness of the product. Increased screw speeds reduced the hardness of the extrudates ($r = -0.79$, $p < 0.01$). A high screw speed was also associated with increased crispiness for the majority of the samples.

Reducing the particle size of rye bran resulted in less hard and crispier extrudates (Publications 1 and 2), (Table 5). Both the crushing force and crispiness work (low Cw: crispier) decreased with particle size reduction, whereas the crispiness index (high Ci: crispier) increased.

Table 5. *Textural properties of the extrudates made of rye bran with varying particle sizes and processing conditions.*

Sample code	Form	Bran category	Hardness (N)	Crispiness work (Nmm)	Crispiness index ($\times 10^{-4}$)
Publication 1: **100% RB extrudates processed at 300 and 500 rpm with 17% IB and PC**					
IB 300	Puff	Coarse	433 ± 56^{f}	16.6 ± 3.2^{f}	0.6 ± 0.1^{ab}
IB 500	Puff	Coarse	245 ± 30^{bc}	6.8 ± 1.6^{bcd}	0.5 ± 0.1^{a}
PC 300	Puff	Coarse	362 ± 47^{e}	8.0 ± 2.2^{cde}	1.6 ± 0.4^{bc}
PC 500	Puff	Coarse	210 ± 42^{ab}	5.4 ± 1.1^{abc}	2.8 ± 0.8^{d}
IB 300	Puff	Medium	464 ± 62^{f}	11.4 ± 5.1^{e}	1.1 ± 0.3^{ab}
IB 500	Puff	Medium	293 ± 42^{cd}	6.0 ± 2.0^{abc}	2.9 ± 0.7^{d}
PC 300	Puff	Medium	461 ± 50^{f}	10.5 ± 3.4^{de}	1.1 ± 0.4^{ab}
PC 500	Puff	Medium	245 ± 33^{bc}	7.0 ± 2.0^{bcd}	2.4 ± 0.6^{cd}
IB 300	Puff	Fine	336 ± 61^{de}	4.2 ± 2.7^{ab}	2.7 ± 0.9^{d}
IB 500	Puff	Fine	188 ± 41^{ab}	2.9 ± 0.3^{ab}	7.2 ± 1.3^{g}
PC 300	Puff	Fine	228 ± 46^{bc}	3.9 ± 0.7^{ab}	4.4 ± 0.8^{e}
PC 500	Puff	Fine	145 ± 17^{a}	3.4 ± 0.7^{ab}	5.8 ± 0.9^{f}
Publication 2: **15 and 30% RB extrudates processed at 500 rpm with 17% IB and PC**					
IB 15	Puff	Coarse	104 ± 23^{lmn}	3.3 ± 0.7^{o}	6.5 ± 1.4^{l}
IB 30	Puff	Coarse	116 ± 19^{mn}	2.9 ± 0.6^{no}	6.1 ± 1.3^{l}
PC 15	Puff	Coarse	96 ± 16^{lm}	3.0 ± 0.5^{no}	7.8 ± 1.7^{lm}
PC 30	Puff	Coarse	107 ± 18^{lmn}	3.0 ± 0.6^{no}	6.1 ± 1.3^{l}
IB 15	Puff	Fine	105 ± 15^{lmn}	2.6 ± 0.7^{mn}	10.7 ± 2.8^{m}
IB 30	Puff	Fine	123 ± 32^{n}	1.7 ± 0.4^{l}	17.6 ± 3.5^{n}
PC 15	Puff	Fine	92 ± 17^{l}	2.5 ± 0.7^{mn}	11.1 ± 3.4^{m}
PC 30	Puff	Fine	98 ± 21^{lm}	2.2 ± 0.6^{lm}	10.7 ± 2.7^{m}
Publication 3: **100% RF (RB-0) and 10% RB (RB-10) puffs and flakes**					
RB-0	Puff	n/a	26 ± 0.3^{r}	0.6 ± 0.1^{r}	258 ± 13^{u}
RB-10	Puff	Fine	30 ± 1.0^{s}	0.7 ± 0.1^{s}	184 ± 29^{t}
RB-0	Flake	n/a	1868 ± 80^{t}	43 ± 3.1^{t}	0.00638 ± 1.0^{s}
RB-10	Flake	Fine	2107 ± 97^{u}	50 ± 4.0^{u}	0.00421 ± 0.4^{r}
Publication 4: **100% RF (RB-0), 40% native RB (RB-40) and 40% fermented RB (FB-40) extrudates**					
RB-0	Puff	n/a	36 ± 4.7^{y}	1.6 ± 0.3^{y}	47 ± 12^{y}
RB-40	Puff	Coarse	49 ± 5.5^{z}	2.2 ± 0.3^{z}	23 ± 4^{x}
FB-40	Puff	Coarse	19 ± 2.3^{x}	$0.48 \pm 0.1_{x}$	430 ± 101^{z}

Values marked with the same letters (a – g; in Publication 1), (l – o; in Publication 2), (r – u; in Publication 3) and (x – z; in Publication 4), for each of the textural properties (hardness, crispiness work and crispiness index) were not significantly different ($p < 0.05$).

The textural properties of the extrudates made with 15% and 30% rye bran supplementation (Publication 2) are shown in Table 5. A reduction in the bran particle size had no significant effect on the extrudate hardness. However, both the crushing force ($r = 0.83$, $p < 0.05$) and crispiness work ($r = 0.85$, $p < 0.01$) of the extrudates were positively correlated with the particle size indicating that fine rye bran extrudates were less hard and crispier (high Ci) compared to the extrudates made of coarse rye bran. These results were in-line with Publication in which rye bran was extruded as

such (Publication 1). The results showed that a reduction of the bran particle size enabled higher bran incorporation in the flour without compromising the product crispiness. A combination of fine rye bran and an IB hydration regimen improved the textural properties of the extrudates particularly for 30% bran addition level. Although IB-30% fine rye bran extrudates were somewhat hard, they had the highest crispiness compared to others.

Puffs were in general less hard and less crispy compared to flakes (Publication 3). Rye bran addition increased the hardness and reduced the crispiness in both puffs and flakes (Table 5). It was clear from the results that bran addition had a negative impact on the textural properties. However, the negative influence of the bran addition was reduced due to use of fine rye bran.

The incorporation of 40% coarse rye bran (RB-40) into endosperm rye flour resulted in hard extrudates. Extruding with fermented rye bran at the same supplementation level (FB-40) reduced the hardness to levels even lower than the extrudates with no added rye bran (RB-0). The fermentation of the bran significantly ($p < 0.05$) increased the crispiness of FB-40 compared to both RB-0 and RB-40. According to the sensory panel, RB-40 were perceived to be the hardest and least crispy extrudates ($p < 0.05$), whereas FB-40 and RB-0 were similar concerning both their perceived hardness and crispiness. RB-40 was perceived to be coarser than FB-40 even though they both had a similar degree of sliminess.

In general, rye bran addition resulted in inferior macro, micro and textural properties. However, bran modification either by particle size reduction or by fermentation improved both the structural and textural properties. Along with the particle size, extrusion-process parameters such as a high screw speed and IB water feed improved the overall quality attributes of rye extrudates.

5.2 The role of extrudate structure and texture on mastication and bolus properties

5.2.1 The effects on mastication properties (Publications III–IV)

Puffs and flakes were significantly different in terms of all mastication properties. Puffs required less oral processing than the flakes (Publication 3), which was reflected by the significantly lower ($p < 0.05$) values for the number of chews, chewing time, applied force/chew and the total work (Table 6). The mastication properties were different only when the structural and textural differences were genuinely diverse such as RB-0 puffs (hardness: 26 N) vs. RB-0 flakes (hardness: 1868 N). Puffs resulted in a paste-like looser bolus structure than the flakes. Rye bran addition to either puffs or flakes did not make any change to the mastication properties compared to the control

extrudates without rye bran. The average saliva uptake of the masticated puffs was 24% higher compared to the flakes. The saliva uptake was negatively ($p < 0.05$, $r = -0.42$) correlated with the hardness and positively correlated ($p < 0.05$, $r = -0.40$) with the crispiness of the products. However, large differences in the saliva uptake between different subjects were observed.

Table 6. *Mastication properties of extruded products made of 10 % (Publication 3), 40% native and 40% fermented (Publication 4) rye bran with varying structural properties.*

Samples code	Form	Number of chews	Chewing time (s)	Force/chew (%)	Total work (%)	Saliva uptake (%)
[1]***Publication 3:*** **100% RF (RB-0) and 10% RB (RB-10) puffs and flakes**						
RB-0	Puff	18 ± 6 [a]	12 ± 4 [a]	191 ± 69 [a]	215 ± 119 [a]	47 ± 10 [a]
RB-10	Puff	16 ± 6 [a]	11 ± 4 [a]	194 ± 70 [a]	193± 92 [a]	45 ± 10 [ab]
RB-0	Flake	29 ± 10 [b]	19 ± 7 [b]	248 ± 74 [b]	438 ± 176 [b]	38 ± 7 [ab]
RB-10	Flake	30 ± 7 [b]	20 ± 6 [b]	255 ± 78 [b]	473 ± 174 [b]	37 ± 9 [b]
[2]***Publication 4:*** **100% RF (RB-0), 40% native RB (RB-40) and 40% fermented RB (FB-40) extrudates**						
RB-0	Puff	11 ± 4	7.8 ± 3	76 ± 21	30 ± 13	43 ± 7
RB-40	Puff	13 ± 6	8.4 ± 4	79 ± 25	34 ± 15	43 ± 7
FB-40	Puff	11 ± 5	7.2 ± 4	71 ± 24	26 ± 13	47 ± 9

[1]*Normalized to the corresponding values of a reference product i.e. white wheat bread (Publication 3).*

[2]*Normalized to the corresponding values of a reference product i.e. chewing gum (Publication 4).*

Values marked with the same letters (a–b) in the same column were not significantly ($p < 0.05$) different (Publication 3). The mean differences were NOT significant at the 0.05 level (Publication 4).

Similarly, neither the addition of 40% native (RB-40) nor 40% fermented (FB-40) rye bran caused any changes to the mastication properties compared to 100% endosperm rye flour (RB-0) extrudates (Publication 4) (Table 6). Rye bran addition had no effect on the saliva uptake, whereas fermented bran increased the saliva uptake in the bolus (visual observation only). The impact of structural and textural differences within the three extrudates was not observed in the mastication properties due to the low statistical power associated with inter-individual variations (Publication 4). The results of this work showed that the subjects were unable to detect subtle structural and textural differences (Publications 3 and 4). High inter-individual variation was observed within fifteen (Publication 3) and twenty-six (Publication 4) individual participants.

5.2.2 The effects on bolus properties (Publications III–IV)

The number of small particles was higher in the bolus samples obtained from puffs than flakes (Figure 9), which was confirmed by the data plotted in granulometric curves (Figure 4, publication 3) and in visual examinations of the photographs (Figure 10). Bolus samples collected from bran added samples had larger particles, but a yet

higher proportion of small particles compared to control (RB-0) puffs (< 10 mm^2: 58% vs 47%) and flakes (< 10 mm^2: 46% vs 39%). The flakes had a greater share of large particles irrespective of bran addition compared to the puffs (39 and 48% of the flake particles were larger than 100 mm^2, for RB-0 and RB-10 samples, respectively, whereas 29% of the puff particles were larger than 100 mm^2 for RB-0 and RB-10 samples).

The average particle area of the puffs was 0.33 and 0.57 mm^2 for the RB-0 and RB-10 samples, respectively. On the other hand, the average particle area of the flakes was 0.52 and 1.13 mm^2 for the RB-0 and RB-10 samples, respectively. The total particle area of the bolus samples was positively correlated with the hardness ($r = 0.99$, $p < 0.05$) and crispiness work ($r = 0.99$, $p < 0.01$), indicating that the harder and less crispy samples resulted in larger particles in the bolus. The number of disintegrated particles increased ($r = -0.96$, $p < 0.05$) when the starch content of the extruded samples decreased by 5% due to bran addition.

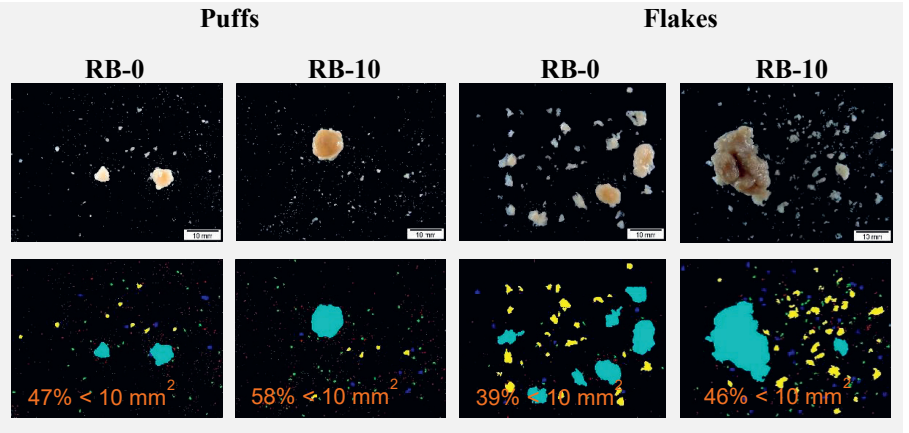

Figure 10. *Row 1: Photographs of masticated bolus samples of RB-0 puffs & flakes and RB-10 puffs & flakes (the white bar is 10 mm) imaged for particle size analysis. Row 2: photographs of calculated bolus samples. The colours indicate: red = 0.001–0.2 mm^2, green = 0.2–0.5 mm^2, blue = 0.5–1.0 mm^2, yellow = 1.0–10.0 mm^2 and cyan blue = 10–1000 mm^2.*

Extrudates with 40% fermented rye bran (FB-40) had a very fragile and crispy texture. As a result, FB-40 extrudates disintegrated easily during mastication and yielded a larger number of small particles compared to RB-0 and RB-40 (Figure 11). The largest particle obtained in the bolus samples were 218, 283 and 142 mm^2 for the RB-0, RB-40 and FB-40 extrudate samples, respectively (Figure 5, Publication 4). The proportion of smaller particles (< 10 mm^2) in the RB-0 (≈ 68%) and RB-40 (≈ 77%) bolus samples were lower compared to the FB-40 (≈ 89%) bolus. However, the average particle area of all bolus samples was between 0.03 and 0.04 mm^2. A strong positive correlation ($r = 0.99$, $p < 0.05$) between insoluble DF and the total particle area indicated that the number of particles in the bolus increased with rye bran addition, which increased the total surface area.

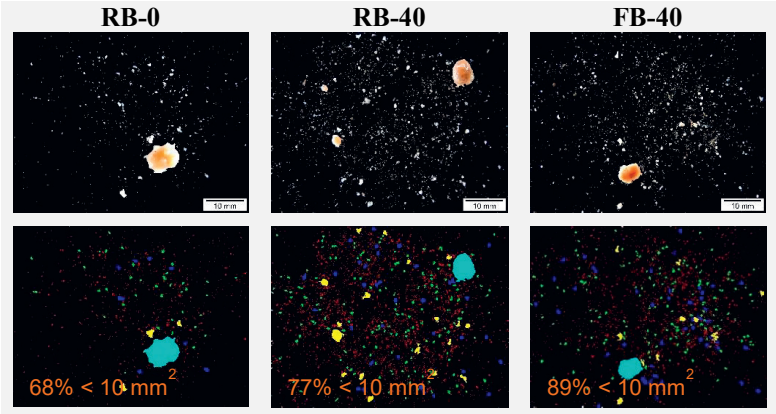

Figure 11. *Row 1: Photographs of masticated bolus samples of RB-0, RB-40 and FB-40 extrudates (the white bar is 10 mm) imaged for particle size analysis. Row 2: photographs of calculated bolus samples. The colours indicate: red = 0.001–0.2 mm^2, green = 0.2–0.5 mm^2, blue = 0.5–1.0 mm^2, yellow = 1.0–10.0 mm^2 and cyan blue = 10–1000 mm^2*

5.3 Starch digestibility of rye bran extrudates

5.3.1 Impact of rye bran concentration and particle size on *in vitro* starch digestibility (Publications I–II)

Extrudates with 100% fine rye bran had significantly a lower HI compared to the extrudates with 100% coarse rye bran (Table 4, Publication 2). However, the effect was reversed at lower bran addition levels. For example, 15% fine rye bran extrudates had a significantly higher HI than 15% coarse rye bran. The effect of the particle size on the HI was not significant at a bran addition level of 30% even though fine rye bran had a 3% higher HI than coarse rye bran extrudates. Surprisingly, 100% fine rye bran extrudates had an identical HI compared to 30% fine rye bran extrudates. In contrast, 100% coarse rye bran extrudates had a significantly higher HI than 30% coarse rye bran extrudates. Nevertheless, all extruded products had lower starch digestibility than wholegrain rye bread regardless of the starch amount, bran addition level and particle size.

5.3.2 The effects of structural breakdown and bolus viscosity (Publications III–IV)

Extruded products with open and closed structures

The extruded puffs and flakes had a high degree of starch digestibility (Figure 5, Publication 3). The addition of rye bran had a tendency to increase the starch digestibility. Puffs and flakes with added rye bran (RB-10) had a significantly higher HI than those made with 100% endosperm rye flour (Figure 5, Publication 3).

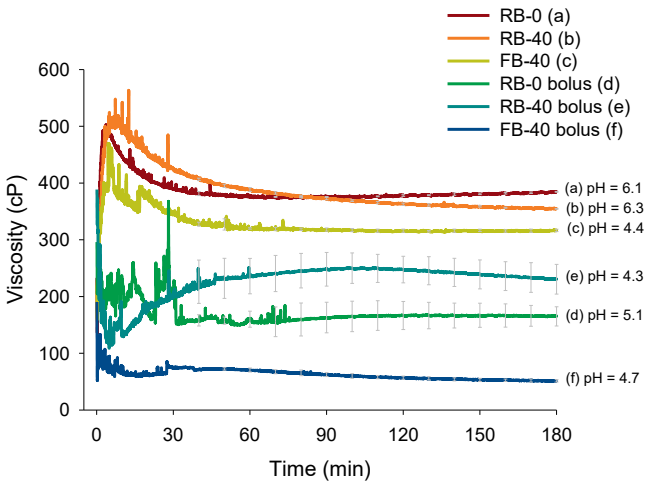

Figure 12. *Viscosity of ground (a, b and c) and bolus (d, e and f) samples of RB-0, RB-40 and FB-40.*

Extruded products with similar compositions but different structures

The fermentation of rye bran significantly increased the *in vitro* starch digestibility of the FB-40 extrudates (Figure 6, Publication 4). An increased rate of starch hydrolysis of FB-40 was observed even at an early phase of enzymatic incubation. After 30 minutes of enzymatic incubation, the starch hydrolysis of FB-40 was found to be 9–13% higher compared to both the RB-0 and RB-40 extrudates. The extrudates with TDF contents of 8% (RB-0) and 22% (RB-40) had a similar hydrolysis index (Figure 6, Publication 4). However, the incorporation of 40% fermented rye bran increased the TDF contents to a similar level of the 40% native rye bran but resulted in a 10% higher HI. Therefore, the differences in the structural and textural features and/or bolus viscosity (Figure 12) were more effective at changing the starch hydrolysis rate than the fibre or starch content.

6 Discussion

6.1 The effects of milling and extrusion on extrudate properties

6.1.1 Composition of dietary fibre

Rye bran particle size reduction increased the amount of SDF but reduced the IDF and TDF contents. During milling, the IDF components such as hemicellulose, cellulose and lignin might have degraded into small molecular substances, which led to an increase in the SDF fraction. Milling the wheat bran also has increased the SDF content due to the degradation of insoluble fibre contents (Zhu et al., 2010).

Extrusion processing had no significant effect on the DF composition of coarse rye bran. However, in contrast to milling, extrusion processing generally increased the IDF and TDF contents of medium and fine rye bran. The TDF contents of the extrudates mainly increased due to the increase of the IDF content, which could be attributed to the formation of polysaccharide and lipid complexes or resistant starch (Gualberto et al., 1997). A high amylose content has been shown to increase the formation of resistant starch (i.e. retrograded amylose) and in turn increase both the IDF and TDF contents (Esposito et al., 2005; Rashid et al., 2015; Stojceska et al. 2009; Vasanthan et al., 2002). Resistant starch is insoluble in nature and thus is part of IDF. However, the effect of extrusion processing on the DF component depends on the processing parameters such as the screw speed, temperature, moisture content, as well as the particle size of the feed materials (Robin et al., 2011b; 2012c).

The solubilisation of IDF as an outcome of high shearing forces and temperature significantly increased the SDF content in some but not in all rye bran extrudates. Extrudates with medium and fine rye bran had higher amounts of SDF, especially those processed with a PC hydration regimen. Particle size reduction by milling, and a PC hydration regimen increased the water absorption capacity of the bran particles. It could be assumed that a PC hydration regimen allowed more time for the bran particles to hydrate and caused more swelling of the bran material. Therefore, the degradation of polysaccharides and protein was probably accelerated for well-hydrated bran particles under high shearing forces and at high barrel temperature. As a result, water soluble small molecular weight components increased due to the degradation of polysaccharides and protein in the extrudates that underwent PC, and this caused higher SDF contents. Moreover, a PC hydration regimen is likely to cause more complete gelatinization of starch, degrading the starch to form soluble polysaccharides under severe extrusion conditions. The results suggest that a PC hydration regimen can improve the functionality of the rye bran by providing sufficient time for water incorporation in high fiber systems. It has earlier been shown that high feed moisture contents and a high screw speed favour the degradation of cell wall material of wheat bran extrudates (Zhang et al., 2011). The DF solubility of wheat

bran was also increased with high extrusion temperature. However, an overly high temperature and low screw speed may result in burning the feed material and lead to a decrease in the SDF content.

Thermo-mechanical decomposition or fragmentation of major insoluble fibre components, i.e. cellulose, hemicellulose and lignin, has earlier been shown the principal reason for increased SDF in extruded (50 rpm, 90−140 °C, and 20−50% feed moisture) barley flour (Vasanthan et al., 2002). Extrusion processing of wheat bran at high screw speeds and temperatures (400 rpm, 140 °C) has also been shown to increase the SDF due to the degradation of polysaccharides glycosidic bonds under mechanical stress and leading to the release of oligosaccharides such as sugar (Esposito et al., 2005). Mild extrusion conditions such as low screw speeds, moderate temperatures and high feed moisture contents (150−300 rpm; 40−160 °C and 10−45% feed moisture) has also been shown to increase the SDF but reduce the IDF and TDF in wheat bran, oat bran and sugar beet fibre extrudates (Gajula et al., 2008; Lue et al., 1991; Zhang et al., 2011). However, in this work high screw speeds, moderate temperatures, and a moderate feed moisture (500 rpm, 130 °C and 17% feed moisture) increased the SDF without reducing the TDF contents.

The fermentation of rye bran solubilized the bran and redistributed the IDF to SDF without affecting the TDF content compared to native rye bran extrudates. Supporting results were also found in a recent study (Nikinmaa et al., 2020) in which rye bran fermentation prior to extrusion reduced the IDF (13 → 11%) and increased SDF (5 → 7%) but did not alter the TDF (18 → 18 %) content compared to native rye bran extrudates. The increase in SDF was probably due to the production of dextran, which is a highly soluble polysaccharide (i.e. SDF). On the other hand, non-starch polysaccharides of DF probably broke down due to acid-induced activation of endogenous xylanolytic enzymes, which caused a reduction in the IDF. Moreover, the decrease in the IDF contents in fermented rye bran extrudates may be caused by the partial degradation of cellulose and hemicellulose into simple carbohydrates i.e. monosaccharides. The ability of fermentation to increase the SDF and reduce the IDF has also been previously reported for oat, barley and soybean DF (Lambo et al. 2005; Tu et al., 2014).

6.1.2 Structure and texture

Macrostructural properties

In this work increased bran concentrations reduced expansion and increased the piece density of the extrudates. Despite a high DF content (27–31%), rye bran exceptionally contains a substantial amount of starch (38–44%) that yields reasonably expanded structures without any further starch addition. The lowest expansion and highest density was obtained for rye extrudates made solely with rye bran. The structural properties improved when the bran addition was kept at 10% and mixed with corn

starch. High amounts of DF in the extrusion feed material reduce the elastic properties of the melt, which in turn leads to a poor gas-holding capacity (Sozer & Poutanen, 2013). Starch plays an important role in building the extrudate matrix and in its expansion. Usually a good expansion during extrusion requires high amounts of starch and low amounts of DF in the feed material (Robin et al., 2011a, b). Extruded products expand sufficiently when the starch and DF contents in the feed material remain between 60–70% and 10–15%, respectively (Riaz, 2000; Sozer & Poutanen, 2013).

DF particles tend to rupture premature air cells (Guy, 1985), which could explain the lower expansion and higher piece density of DF-rich extrudates. Moreover, due to its high-water biding capacity, DF competes for water with starch and eventually causes incomplete starch gelatinization (Camire & King, 1991). Increased amounts of bran may lead to a higher amount of free water for the starch phase, which reduces the glass transition temperature of the starchy melt (Robian et al. 2011a, b). Therefore, a higher percentage of bran/DF hinders the radial expansion and results in a dense structure. Bran from different sources, for example, wheat, corn and oat, and apple pomace have also been found to reduce the radial expansion and increase the piece density and specific length (Brennan et al., 2008a, b; Robin et al., 2012a; Sibakov et al., 2015).

In this work, supplementation of 10–30% rye bran in endosperm flour resulted in 74–82% starch and 8–13% DF and therefore increased the expansion and reduced the density compared to extrudates made solely of rye bran. Higher amounts of bran resulted in a lower expansion rate and a higher piece density in both puffs and flakes. The detrimental impact of rye bran was more drastic when the bran percentage was increased to 40%. The addition of 40% rye bran significantly reduced the starch (57%) and increased the TDF (22%) content of the extrudates. The addition of more than 20% wheat or oat bran (i.e. 26 and 32%) into whole wheat and corn based extrudates has also been shown to cause significant loss of expansion (Chanvrier et al., 2014).

Higher screw speeds (500 vs 300 rpm) increased radial expansion and specific length but reduced piece density of the rye bran supplemented extrudates, which is in accordance with studies performed earlier with corn meal-soy fibre extrudates (Guha et al., 1997; Jin et al., 1994). High screw speed probably decreases the melt viscosity due to the increased shear force inside the extruder barrel and therefore the specific length increases (Jin et al., 1994). On the other hand, high screw speeds increase the frictional heat and further increases the product temperature, which allows a higher number of starch granules to be dispersed into a polymer phase resulting in increased expansion and reduced piece density (Jin et al., 1994). In this work extrudates with a higher in-barrel water feed resulted in lower expansion, which is in agreement with the study by Liu et al. (2011) who observed that a higher IB water feed usually resulted in reduced expansion of extruded moringa leaf and oat flour snacks. A higher IB water feed is associated with lowering the viscosity by reducing the melt temperature, which coincides with more cell collapses. However, inconsistent effects of the hydration regimen on expansion were found in this work for coarse-particle sized bran, and pre-

conditioning counter affected and reduced expansion. One reason for this might be that the coarse-particle-sized bran bound more water during preconditioning due to its greater water binding capacity, thus reducing the water amount available for expansion. Neither the specific length nor the piece density were affected by variation in the hydration regimen.

Microstructural properties

Light microscopy images comparing native and extruded rye bran showed that high shear forces during extrusion resulted in mechanical degradation of the bran structure. The effect was less severe in coarse and medium compared to fine rye bran extrudates. The rye bran particles were aligned within the air cell walls of the extrudates, where large bran particles interfered with radial and longitudinal extensibility.

Extrudates with 100% rye bran were less porous and had a low average cell diameter, but reducing the bran concentration (10–30% of the feed) increased both the porosity and the average cell size. However, the addition of 10% rye bran had no significant effect on the microstructural properties of both puffs and flakes compared to the products without bran, which was most likely due to the low addition levels. The adverse effects of rye bran on the microstructural properties of the extrudates were possibly compensated for by the relatively low bran addition level (10%) and the fine particle size (24 μm). Similar effects of particle size reduction on the microstructure have also been obtained for oat bran extrudates, where an oat bran concentrate (37 um) addition did not interfere with the structure as long as the addition level did not exceed 10% of the feed (Sibakov et al., 2015).

Increasing the bran amount from 15 to 30% had no impact on the porosity and average cell diameter when rye bran was incorporated into a blend of rye flour and corn starch. This finding contradicts earlier work by Chanvrier et al. (2014) in which wheat and oat bran concentrate (10−32%) supplementation in a blend of whole grain wheat-refined and wheat-corn flours gave extrudates with TDF contents varying between 5–17 %, which resulted in a low average cell diameter and porosity. However, in this work, the highest TDF content was 12.6% with a 30% bran addition which was much lower than in Chanvrier et al. (2014). Therefore, it could be hypothesised that both the low TDF and proportionally higher starch content in this work did not affect the microstructural properties.

Both macro- and microstructural properties were well correlated. For example, porosity correlated positively with expansion ($r = 0.97$, $p < 0.01$) and negatively with piece density ($r = -0.97$, $p < 0.01$), whereas piece density was found to have a negative correlation with expansion ($r = -0.99$, $p < 0.01$). The relative connectivity of the pores is associated with the fragmentation index (FI) values. Low FI values (higher negative values) indicate more connected structures, whereas the high FI values (lower negative values) represent a more disconnected structure (Hahn et al., 1992; Sozer et al., 2011b). Rye bran extrudates could be concluded to have more connected porous

structures due to negative FI values. However, in this work the fine rye bran extrudates had comparatively more disconnected pores than coarse and medium particle sized bran extrudates.

Textural properties

Extrudates with 100% rye bran were hard and less crispy regardless of the hydration regimen. The incorporation of bran or DF in corn, wheat, rye, and oat extrudates also resulted in a hard and less crispy texture, as reviewed by (Heiniö et al., 2016). However, a high screw speed improved the textural properties for the rye bran extrudates. For example, in this work 100% rye bran extrudates processed at 500 rpm were less hard but more crispy compared to the extrudates processed at 300 rpm. Ding et al. (2006) showed that high screw speed reduces the melt viscosity, which promotes better expansion, lowers piece density and in turn reduces extrudate hardness. However, in this work all extrudates made of solely rye bran in general had hardness values which were above the critical threshold (200 N; Lobato et al., 2011) to be accepted by the consumers. The results of this work indicated that preconditioning of 100% rye bran improved the hardness in all cases regardless of the screw speed and the particle size. However, the effect of preconditioning on the crispiness was somewhat unclear even though preconditioning improved the crispiness in most cases.

In this work the textural properties of rye bran extrudates significantly improved by lowering the bran concentration (15–30%) by adding rye flour to the feed. Similar results were reported in other studies of oat, whole wheat, and wheat flour extrudates, where reducing the wheat and oat bran concentration from 20% to 5% reduced the hardness and increased the porosity and crispiness (Brennan et al., 2008a; Chanvrier et al., 2013; Sibakov et al., 2015). Higher bran concentrations have also been found to increase the cell density and number of pores, which are responsible for a hard and less crispy texture in bran-containing extrudates (Robin et al., 2011a). In this work, the hardness of rye bran extrudates had a negative impact on the porosity ($r = -825$, $p < 0.05$) and on the crispiness index ($r = -0.718$, $p < 0.01$), indicating that extrudates with lower hardness values were more porous and crispier.

As expected, rye bran addition at lower levels (10%) did not lead to any major differences in the textural properties of either puffs or flakes (8% TDF) compared to the extrudates with no bran. Thus, this bran addition level would create palatable extruded products with added nutritional value without compromising structural or textural properties. Similar results were reported earlier with oat extrudates (7% TDF), where no significant changes in textural properties were observed with the addition of 10% oat bran in defatted oat flour extrudates in the study by Alam (2012). In this work the textural properties of puffs and flakes were noticeably different, even though both had the same matrix composition. The flakes were very hard and less crispy compared to the puffs with or without bran addition. The interaction of the structural and textural properties of puffs and flakes showed that puffs and flakes with lower average cell

diameters were less crispy and hard. Similar results were obtained for 100% rye bran extrudates, where extrudates with smaller air cells were harder and less crispy.

6.2 Modification of bran to improve extrudate properties

6.2.1 Bran particle size reduction

Particle size reduction from 440 µm to 28 µm resulted in improved expansion, decreased hardness, and increased crispiness for extrudates made of solely rye bran. In previous studies, it has been shown that a reduction of the particle size of corn bran (50 µm), corn flour (1622 → 50 µm), corn grits (518 → 101 µm), and wheat bran (702 → 84 µm) also resulted in significant increases in expansion, and resulted in less hard and crispy extrudates (Garber et al., 1997; Desrumaux et al., 1998; Pai et al., 2009; Santala et al., 2014). Large fibre particles restrict the development of air cells by disrupting the air cell walls during the nucleation phase of the cells, which causes air cells to collapse before the most favourable expansion takes place. Therefore, feed materials containing coarse particles usually result in less expanded products. On the other hand, fine particles help reduce the possibility of early ruptures of the air cells and therefore reduce air cell collapse before their optimal expansion (Lue et al., 1991). Fine particles propagate a large number of air cells by providing more nucleation sites and this results in a better expansion (Lue et al., 1991). A reduction of the particle size also increases the hydration capacity of bran and therefore the melting time required for fine bran is shorter (Desrumaux et al., 1998; Santala et al., 2014; Sozer & Poutanen, 2013). Hence, the starch transformation becomes higher with finer particles, which in turn improves the macro-, micro- and textural properties of the extrudates.

The particle size of the bran plays an important role in forming the cellular structure of the extrudates as bran particles become entrapped by the continuous starch matrix. Therefore, the possibility to achieve an expanded product increases when the particle size of the bran is reduced to a certain level to promote foam expansion (Guan et al., 2004). In order to gain the beneficial effects of particle size reduction on expansion the average particle size of the bran must be below 100 µm and should be added at substantially high levels to the feed material (more than 15%). For example, for wheat bran, a reduction from 317 to 224 µm was found not to increase expansion (Robin et al., 2011b). Additionally, the addition of 10% defatted oat bran concentrate (213 → 32 µm) has been found not to change the expansion rate of defatted oat endosperm flour extrudates due to relatively low bran supplementation levels (Alam, 2012).

In this work highly porous structures were obtained with fine rye bran. The starch matrix was less disrupted in fine bran extrudates, which reduced cell growth failures. Early ruptures of the precursor air cells resulted in a higher small cell count in medium and coarse bran extrudates. Similar results were reported for wheat extrudates, where

coarse wheat bran has been shown to produce higher numbers of small cells (Robin et al., 2011b; 2012b). In this work coarse and medium rye bran disrupted the continuous structure of the starchy matrix more frequently and created a weak adhesion sites within the viscous melt. As a result, a higher number of small cells were formed in coarse and medium rye bran extrudates, which is in accordance with the study by Karkle et al. (2012). This work found that extrudates processed with coarse rye bran had small homogeneous air cells, whereas extrudates with fine bran had large but inhomogeneous air cell distribution. Extrudates made of fine (2000 → 74 µm, 20−30%) wheat bran, sugar beet fibre and oat fibre have also been shown to have a higher number of large air cells, and thus favour expansion compared to the coarse fibre (Lue et al., 1990; 1991).

This work achieved a high level of crispiness with 30% fine rye bran processed with an in barrel water feed compared to others with 15, 30 and 100% rye bran processed under different extrusion conditions. In this case, the cell area was higher compared to the others, which probably increased the number of peaks (i.e. frequency of spatial ruptures) in the force-deformation curve and resulted in a crispy texture. It has earlier been shown that increases in the cell diameter increased the number of peaks in the force-deformation curve and therefore increased the crispiness (Karkle et al., 2012). However, the results of this work showed that the combination of high screw speeds and fine bran further increased expansion, reduced the hardness, and increased the crispiness.

6.2.2 Bran fermentation

The incorporation of 40% fermented rye bran into endosperm rye flour in this work significantly increased the expansion and crispiness and reduced the extrudate hardness and density compared to extrudates with native rye bran with a similar composition. The effect of fermentation was more pronounced on the textural properties than on the expansion. For example, the crispiness index of the fermented rye bran extrudates increased by nine- and nineteen- fold compared to the extrudates made of 100% rye flour and flour with 40% native rye bran, respectively. The expansion rate of the studied extrudates with fermented rye bran was slightly lower (414 vs 525%) than 100% rye flour extrudates. Despite the different expansion rates, extrudates with 40% fermented rye bran and those made of 100% rye flour were fairly similar in terms of the piece density. These results were in line with the perceived sensory properties, where the trained sensory panel in this work detected a large textural difference between fermented and native rye bran extrudates, but no difference was detected between 40% fermented and 100% rye flour extrudates. It has also been shown in a recent study that rye bran fermented with *Weisella confusa* in the presence of 10% sucrose efficiently increased the expansion rate (489 vs 422%) and the crispiness index (0.002 vs 0.06) but reduced the hardness (16 vs 43 N) and density (99 vs 166 kg/m^3) compared to native rye bran extrudates (Nikinmaa et al., 2020). The

same study also showed that chemically acidified rye bran without fermentation increased the expansion and crispiness but reduced the hardness and density to similar levels to those that have been achieved with fermented rye bran extrudates (Nikinmaa et al., 2020). Hence, improved macrostructural and textural properties of fermented rye bran extrudates have been achieved not only due to the effect of dextran but also through fermentation-induced acidity in fermented bran.

However, in this work dextran produced *in situ* during the fermentation of rye bran probably formed a weakly bonded association with DF components and proteins. As a result, the overall viscosity was significantly affected in the final product. Although it is not yet clearly known how EPS fermentation influences product quality, several hypotheses could explain the changes in structural and textural properties.

Firstly, the fermentation of rye bran with EPS-producing *Weissella confusa* could have possibly created a layer of dextran on the surface of the bran particles, which smoothened the bran surface and therefore reduced premature air bubble ruptures, as described by Nikinmaa et al. (2017). Secondly, dextran produced by *in situ* fermentation has been shown to have the ability to influence a product's texture due to the capability to affect the viscosity (Lambo et al., 2005). Thirdly, the solubilization of IDF might have improved the structural and textural properties (Katina et al., 2007; Pai et al., 2009; Santala et al., 2014). It has also been shown that the fermentation of rye bran with dextran- producing strains increased the soluble pentosans in rye bran (Katina et al., 2007; Nikinmaa et al., 2017). Finally, and most importantly, acid production during fermentation might have activated proteolytic and xylanolytic enzymes, which hydrolyses protein and DF (Nikinmaa et al. 2020). In this work the water binding capacity of DF and protein was probably reduced due to acid hydrolyzation. As a result, more water was available for starch during extrusion and therefore enhanced starch gelatinization. In addition, a low pH probably induced starch hydrolysis, which might also have an impact on the starch gelatinization. Furthermore, rye proteins have a high foaming capacity, and the foam remains stable even after heating. Therefore, solubilized rye bran protein due to acid hydrolysis might have a foam stabilizing effect, which also partly explains the better structural properties of fermented rye bran extrudates.

In this work, IDF was decreased by 13% and SDF was increased by 33% in fermented rye bran extrudates compared to the unfermented counterparts. There might be a degradation of insoluble polysaccharides or redistribution of IDF to SDF in fermented rye bran extrudates. Similar results were reported for barley DF, where the IDF content decreased by 56% and SDF content increased by 50% after fermentation with dextran- producing strain (Lambo et al., 2005). It has been reported that SDF has the ability to improve the functionality of DF in extruded products (Brennan et al., 2008a; b; Robin et al., 2012c; Yanniotis et al., 2007). In earlier work, the SDF content in corn bran increased due to the solubilization of IDF, which had a positive impact on expansion compared to untreated bran (Pai et al., 2009). Increased amounts of SDF

induced favourable changes in the melt viscosity and implement better interaction between SDF and starch (Pai et al., 2009).

Dextran produced by *in situ* fermentation has been shown to influence the texture of bread due to its capability to affect the viscosity (Katina et al., 2009). A small amount of *in situ* dextran (1–2%) in wheat sourdough has been shown to be capable of improving the volume of bread and crumb softness by 10–40% (Katina et al., 2009). However, in bread dough, the viscosity was increased due to dextran formation. Additionally, extrusion feed material (bran-flour mixture) containing dextran has also been found to have a higher final viscosity compared to native bran (Nikinmaa et al., 2020). However, in this work, the viscosity of the feed material was not studied. On the other hand, the viscosity of the extrudates was lower for the fermented bran extrudates. Perhaps the depolymerization of starch due to the acidic environment caused a lower viscosity in the fermented rye bran extrudates. In earlier work, increased acidity of cassava extrudates has been shown to improve the expansion rate (Sriburi & Hill, 2000). Therefore, the adverse effect of native rye bran on the structural and textural properties of extrudates could be reduced by using fermented (3% dextran) rye bran, even though it is not known where the positive effect of bran fermentation comes from, whether it arises from the increased acidity or whether it is due to the dextran production.

6.3 Mastication and bolus formation of extrudates

6.3.1 Mastication behaviour

In this work softer and crispy puffs with a high initial moisture content were easy-to-break and therefore required less mastication effort compared to hard and crunchy flakes with less moisture. Flakes required a higher mastication effort not only for particle breakdown but also for insalivation. It has been shown in earlier studies that an extended chewing time may not always reduce the particle size, but it may allow more time for the agglomeration of the fractured particles and saliva uptake to form a swallowable bolus (Fontijn-Tekamp et al., 2000; Jalabert-Malbos et al., 2007; Le Bleis et al., 2016). This is in agreement with our results, as the flakes with a hard texture took a longer chewing time, but still had a greater share of large particles compared to the puffs.

Extruded puffs in this work with no bran or a 10% rye bran addition had a similar mastication profile. It was expected that puffs with added rye bran would require a higher mastication effort, but there were no major differences in the mastication. The reason for this might be that rye puffs with or without rye bran had similar macro- and microstructural properties. However, the effect of the structure on the mastication profile was observed only when there were large structural differences; for example,

puffs and flakes were significantly different during mastication regardless of the rye bran addition.

In this work puffs with 40% fermented rye bran were significantly softer and crispier than native rye bran added puffs with a similar composition. According to the trained sensory panel, the fermented and native rye bran puffs were distinctly different in terms of the textural attributes. Although the puffs with fermented rye bran required a lower mastication time (7.2 vs 8.4), lower total work (26% vs 34%) and a lower force/chew (71% vs 79%) than the puffs with native rye bran, the differences were not statistically significant. Perhaps the high inter-individual variation overrode the possible structural differences between the products.

The instrumental texture analysis used in this work was sensitive, well calibrated, and was performed in a controlled way. As a result, the variations between the products were efficiently detectable even within similar product types with different compositions (i.e. puffs vs rye bran added puffs). However, the differences detected with the texture analysis was not enough to reflect any changes in the mastication properties due to the variation in human chewing patterns and applied force during mastication. Inter-individual variation between the subjects probably masked the differences between the products. We believe that including more subjects in the mastication trials would reduce the high standard deviation that we had in this work. However, increasing the number of mastication subjects from fifteen to twenty-six did not help in reducing the large variation in the results. On the other hand, the differences between the products due to different bran amounts (i.e. puffs vs. rye bran added puffs) was noticeable when the mastication data of the same subject for different products was studied individually. Therefore, it could be seen that the texture of the food had an effect on disintegration in mouth, but the disintegration pattern still varied a lot depending on the mastication behaviour of the individual subjects. A similar phenomenon has been previously reported for different food types such as vegetables, meats, cheese and eggs (Jalabert-Malbos et al., 2007). However, the structural and textural differences for the extruded products examined in this work were not truly reflected in the mastication properties due to high standard deviation.

6.3.2 Bolus properties

In this work softer and crispy puffs easily disintegrated during mastication and produced more particles in the bolus than flakes with a similar composition. An increased number of particles in the bolus increased the total surface area and therefore required more saliva for particle agglomeration and bolus formation. A similar result was reported for crispy bread, where a higher number of small particles required more saliva for lubrication and agglomeration to form a swallowable bolus (Gao et al., 2021; Pangborn & Lundgren, 1977). Dry and porous food require more saliva to soften the structure and to cluster the chewed particles. Porous foods require increased amount of saliva also to fill porous gaps as well the gaps between chewed particles to increase

viscous cohesion (Moska & Chen, 2017). It has earlier been shown that bread required five times more saliva due to its porous microstructure compared to cooked pasta (Hoebler et al., 1998).

During mastication, the food structure is degraded into smaller particles. Sufficient lubrication and surface coating of the particles is important. Saliva produced during mastication clusters the food particles together and prevents them from sticking to the oral surfaces or between the teeth. Insufficient saliva may result in an uncomfortable dry feeling in the mouth, which is often observed during the consumption of dry, crispy, and easily fractured foods (Moska & Chen, 2017). However, in this work, large variations in the saliva uptake of the subjects caused a high standard deviation in the mean value. As a result, there were only minor differences in the average saliva uptake, which was not sufficient to cause any statistical differences. Saliva produced during oral processing of food is an automatic process and therefore cannot be controlled. The amount of saliva secretion depends both on the individual and the type of food eaten (Moska & Chen, 2017).

The textural properties of food products such as the hardness have a significant effect on mastication and bolus formation. The incorporation of 10% rye bran in this work increased the hardness and reduced the crispiness in both puffs and flakes, which resulted in an increased number of bolus particles. In earlier work, increased food hardness also led to higher number of small bolus particles in nuts and cheese compared to soft foods such as jelly and peaches (Chen et al., 2013). However, the effect of the hardness on mastication was the opposite in this work when puffs and flakes were compared, regardless of rye bran addition. The expanded and porous structure of puffs with high initial moisture made them easily breakable compared to the flakes, which affected the overall mastication behaviour and disintegration patterns. For example, it has been reported that white wheat bread with a high initial moisture content (44 vs 22%) required a shorter chewing cycle to produce a ready-to-swallow bolus compared to cake with a low moisture content (Motoi et al., 2013).

An increased number of particles in the bolus was also observed with the addition of 40% of either native or fermented rye bran in this work compared to control extrudates without rye bran. The incorporation of a higher amount of IDF by adding rye bran made the extrudates less cohesive, which might have restricted cluster formation and led to the formation of smaller particles in the resulting bolus. Particle disintegration further increased in fermented rye bran extrudates due to their less dense and crispy texture. Despite a similar percentage of rye bran, fermented rye bran extrudates resulted in a greater number of fine particles in the bolus compared to the native counterparts. The sensory panel also confirmed that the fermented rye bran extrudates were perceived as less coarse than the native rye bran extrudates.

The differences in the mechanical properties of food particles as well as their ability to absorb saliva determine their hydration and swelling properties, in addition to their dissolution, which may result in different bolus rheology (Witt & Stokes, 2015). The

texture of the bolus samples obtained from native and fermented rye bran extrudates in this work was very different. The native rye bran extrudates were more compact and slightly dry with less saliva, whereas the fermented rye bran boluses had a loose, paste-like structure with slightly more saliva. The dissolution of polymers might have increased the adhesion of the saliva to the bolus particles of the fermented rye bran extrudates and resulting a sticky and loose boluses, which is in accordance with the outcome of an earlier study on hard and soft biscuits (Witt & Stokes, 2015). In contrast, comparatively intact starch-protein matrices in native rye bran extrudates absorbed less saliva and resulted in dry boluses for the native rye bran extrudates. Differences in the bolus texture were also observed between puffs and flakes, as the puffs had a sticky texture, but the flakes seemed comparatively dry.

The textural differences may be due to changes in saliva absorption when small fragments of food agglomerated to form the bolus mass (Witt & Stokes, 2015). If the rate of saliva absorption is higher than the rate of saliva formation, the resulting bolus may remain dry, which is due to the depletion of lubricating saliva from the oral surfaces. In contrast, the dissolution of polymers increases the adhesion of the bolus particles to the oral surfaces and in turn produce a sticky bolus texture. However, the absolute moisture contents of the bolus at the swallowing point may not vary a lot for dry foods with initial moisture contents between 3.8 and 8.4 (Loret et al., 2011). This was also the case in our study, where the final moisture content was almost similar.

Inter-individual variation between subjects

In this work there was large variation in the mastication properties and in the saliva uptake of the bolus samples. Therefore, it could be assumed that the dominant source of the variation was also due to inter-individual variability. Large variation between individual participants has also been reported in earlier studies for mastication parameters as well as particle size distribution (Foster et al., 2006; Jalabert-Malbos et al., 2007). Inter-individual variation also affected the particle size distribution; for example, some participants masticated extruded products for longer periods of time and their bolus samples contained numerous uniform small particles. On the other hand, some participants thought that the bolus was ready to swallow even when some particles were significantly large and uneven in shape. It has earlier been shown that the particle size distribution of a bread bolus varied between 0.7 to 2.7 mm and that the variation arose due to inter-individual differences (Le Bleis et al., 2016). Therefore, it was impossible to detect the structural differences between samples during mastication. Large inter-individual variation in this work made it challenging to quantify how structural differences influenced the oral processing of rye extrudates.

6.4 *In vitro* starch digestibility

6.4.1 The effects of rye bran concentration and bran particle size

In this work extrudates made solely of fine rye bran had a significantly lower (68.9 vs 75.4) starch hydrolysis index (HI) compared to coarse bran extrudates, despite the relatively high starch content. Fine rye bran had a 33% higher cell wall thickness than the coarse bran extrudates. As a result, a significant portion of the particles remained large in the ground fine bran extrudate samples, which made it difficult for starch granules to come into contact with enzymes and they thus remained intact. Starch hydrolysis of extruded products depends on the food matrix composition as well as on the status of starch. It has been shown earlier that the structural properties of extrudates also affect the starch digestibility of the processed food (Tovar et al., 2003).

In extrudates where there was a more abundant level of starch, increasing the bran concentration from 15 to 30% reduced HI by 5–7% for both fine and coarse particle sized rye bran extrudates in this work. However, an inverse relationship between the bran concentration and HI was only significant for fine bran extrudates. Coarse bran contains a larger part of the seed coat and the surface area to volume ratio remains lower compared to fine bran. As a result, coarse bran acts as a barrier to enzymic attack and consequently reduces the starch digestibility. In this work, coarse rye bran also reduced the HI (74.6 → 70.3 and 69.0 → 66.9, for 15 and 30% rye bran, respectively) but the effect was only statistically significant for 15% rye bran. It has earlier been shown that increasing the particle size from 800 to 3000 μm reduced the starch digestibility for corn and wheat extrudates, with fibre contents of 2.4% and 3.3%, respectively (Anguita et al., 2006). Interestingly in this work extrudates with 30% fine bran had similar starch digestibility compared to 30% coarse bran. Extrudates with 30% fine bran had a 19% higher cell wall thickness compared to coarse rye bran extrudates. Increased cell wall thickness in fine bran extrudates might have prevented the accessibility of amylases to the starch granules and therefore reduced the starch digestibility to similar levels as those for coarse rye bran.

In this work extrudates with 30% fine bran had identical HI values compared to extrudates made of 100% fine rye bran. Amylopectin rich waxy corn starch was used to produce 30% fine rye bran extrudates, which makes comparison with 100% rye bran extrudates challenging. Amylopectin is more readily digestible than amylose as the branched chain structure hinders its crystallization. Therefore, extrudates with 30% fine rye bran should have had a higher HI but the effect was counterbalanced by the cell wall thickness. However, significant effects of particle size reduction on the starch digestibility was observed at only a 15% bran addition level; when lowering bran particle size increased starch digestibility.

In this work, bran fermentation resulted in a higher HI (104.4 vs 94.4) compared to native rye bran extrudates. Despite a similar bran composition and coarse median particle size (365 μm), a significant increase in the HI was observed with fermented bran only, whereas extrudates made of native rye bran (TDF: 22%) had an almost identical HI compared to extrudates without rye bran (TDF: 8%). This is due to the fact that the starch digestibility not only depends on the composition but also structural or textural differences. The fermented rye bran extrudates had a more expanded and brittle structure, which led to the production of more fine particles during structural breakdown and consequently increased the *in vitro* starch digestibility rate. Rye bran addition did not reduce the starch digestibility in both native and fermented rye bran extrudates. Similar results were reported for rye breads, where increasing the TDF (7 → 12%) by adding rye bran did not reduce the HI compared to rye endosperm flour breads (Rosén et al., 2009). In contrast, high DF rye breads (TDF: 6 vs 29%) were shown to have a lower starch hydrolysis rate compared to endosperm rye bread (Juntunen et al., 2003). However, the accessibility of starch to α-amylases might be different according to the processing methods, ingredients, and bran to flour composition (Pentikäinen et al., 2014).

In general, the starch digestibly of the extruded products was quite high in this work, which is most probably due to the disruption in structural integrity of starch granules caused by high shearing stresses during extrusion. It has been shown that intense mechanical treatment such as a high amount of shearing in the extruder provokes complete degradation of starch at high temperatures, high pressures and with a low water content of the feed (Anguita et al., 2006; Brennan et al., 2012). High starch hydrolysis rates of extruded products have also been reported in many other studies despite their DF content (Altan et al., 2009; Anguita et al. 2006; Brennan et al., 2008b). Disrupted starch granules are likely to be affected more readily by digestive enzymes, thereby enhancing digestion (Brennan et al., 2008b).

It has been shown that extrudates with 5–15% soluble fibre (such as guar gum and inulin) and insoluble fibre (such as wheat bran) had higher starch digestibility (Brennan et al., 2008b, Brennan et al., 2012). However, the guar gum added extrudates had significantly lower starch hydrolysis rate than the wheat bran added extrudates. Soluble fibre increased the hydration capacity and formed a gel type slurry during digestion, which may slow down the starch digestibility (Brennan et al., 2008b). A similar trend of reduced starch digestibility has been shown for other DF sources such as tomatoes and apple pomace up to addition levels of 25% (Altan et al., 2009; Karkle, 2011).

Although the literature suggests that increased SDF contents increase viscosity, it was not observed in this work with dextran- containing fermented rye bran. This was most likely to be due to the acidification of fermented bran, which altered the

functional properties of native starch. It has been shown that acid hydrolysis reduces the swelling capacity of starch granules (Wang & Copeland, 2015).

Amylopectin rich starch is more susceptible to acid hydrolysis than starch rich in amylose (Wang & Copeland, 2015). Rye starch contains a substantial amount of amylopectin compared to other cereal starches and is thus more susceptible to acid hydrolysis. The swelling capacity of acid-hydrolysed waxy corn, rice, and sorghum starch has been shown to reduce after 24 h of hydrolysis, as reviewed by Wang & Copeland (2015). In this work rye bran was fermented for 20 h and the acid produced during fermentation hydrolysed the rye starch and reduced the swelling capacity. In earlier work, acid hydrolysis disrupted the amylopectin chain and resulted in fragile structures and fragmented jack bean (*canavalia ensiformis*) starch granules (Betancur & Chel, 1997). The disrupted amylopectin chain no longer swells because it cannot entrap water but rather is dissolved (Wang and Copeland, 2012). Moreover, acid hydrolysis also produces low molecular weight dextrins (Wang & Copeland, 2012), which tend to dissolve rather than swell during heating in water and consequently resulting in the low viscosity profiles of acid-hydrolysed starches.

A reduced swelling capacity has been shown to increase the pasting temperature and to retard the viscosity development of corn starch (Wang et al., 2003). Therefore, the pasting temperatures of the fermented bran extrudate starch in this work was probably increased due to the acidity induced by fermentation. In this work the viscosity was analysed at a constant 37 °C (to mimic human body temperature), which was quite low and thus starch from the fermented rye bran extrudates could not form a viscous paste.

An increase in viscosity occurs when the majority of the starch granules become swollen. Rupture of the swollen granules causes a decrease in the viscosity. Therefore, another explanation could be that the starch granules of the studied fermented rye bran extrudates leached out and swelled faster due to their mechanically weaker structure and crispy texture, but early ruptures of the swollen granules might have caused a reduction in viscosity.

6.4.2 The effects of bolus particle size

In this work rye bran resulted in a less cohesive structure in puffs and flakes, which prevented cluster formation of numerous small particles during bolus formation. The small bolus particles made the starch more available to digestive enzymes and increased the HI. Degradation of the extrudate matrix either by milling or by chewing increases the total surface area thus leading to a higher degree of starch hydrolysis (Singh et al., 2010; Tovar et al., 2003).

Starch digestibility increased significantly in rye bran added puffs and flakes in this work, although the HI of puffs and flakes without the addition of bran was similar.

The starchy matrix of the puffs and flakes with added rye bran was disrupted due to the presence of insoluble bran particles, which resulted in a higher number of small particles and led to an increase starch hydrolysis rate. This is in accordance with similar results for bran added pasta (Eelderink et al., 2015), where the addition of wheat bran increased *in vivo* glucose responses. The bran addition was the probable cause of small cracks within the matrix of the studied extruded products, which led to increased susceptibility to enzyme attack. In another study it has been shown that finely milled (800 µm) extrusion feed material increased the starch hydrolysis of corn and wheat extrudates (Anguita et al., 2006). In this work the rye bran, which was added to the puffs and flakes, were very finely milled and therefore increased the starch hydrolysis rate.

Adding rye bran to puffs and flakes increased the IDF contents in both puffs (2.8 → 5.2%) and flakes (4.0 → 5.7%). The increased IDF led to a higher number of particles in the boluses of the puffs (718 → 1406 / g of bolus) and flakes (387 → 916 / g of bolus) compared to the puffs and flakes without bran. Similar results were obtained with 40% native rye bran extrudates. The addition of rye bran increased the number of small particles in the bolus compared to the extrudates without rye bran. The proportion of small particles further increased in rye fermented bran extrudates. The presence of a high number of small particles resulted in an increased effective surface area, increasing the accessibility of starch granules to enzymes and allowing the α-amylases to penetrate the internal structures more readily. A positive correlation ($r = 0.85$, $p < 0.05$) between the number of bolus particles and the starch hydrolysis rate was found in this work.

The effect of structure disintegration and particle size on the starch digestibility of different breads (baked bread, steam bread and French baguettes) has been studied in a recent study (Gao et al., 2021). In their study, steam bread containing smaller particles was associated with a lower rate of starch hydrolysis (63% vs 68% and 73%) during gastric digestion compared to baked bread and baguettes, which contradicts the results obtained in this work. However, the particle size was shown to play a significant role in the overall digestion process. The average particle size of the bolus, chime and digesta was 20 mm^2, 109 µm and 73 µm and the corresponding starch hydrolysis rate was 9.5%, 49% and 68%, respectively.

The structure of food plays an important role in determining the digestion properties. The porous structure of wheat bread was shown by Pentikäinen et al. (2014) to have a higher starch digestibility rate compared to dense rye bread. In their study, even though rye breads were disintegrated into smaller particles, the disintegrated particles had a compact structure, whereas wheat bread had large but airy particles. The open porous structure of wheat bread thus favoured interaction between the starch granules and α-amylases and resulted in higher starch hydrolysis, which explains the higher starch digestibility of the porous fermented rye bran extrudates. However, whole rye bread had less starch (35 vs 46) and more DF (11 vs 6) than

endosperm rye bread (Pentikäinen et al., 2014). Despite less starch and a high amount DF, whole rye bread was digested more rapidly than endosperm rye bread, which was probably due to the higher share of small particles (> 1 mm^2: 58% vs 51%; > 10 mm^2: 93% vs 90%) in whole rye bread. The presence of insoluble fibre probably disrupted the starch-protein matrix and produced more small particles, which was also the case in our study, where bran addition increased the number of small particles in the bolus. However, the food type dominates the digestion process more than the oral processing, as the impact of oral processing is diminished over the digestion process from the mouth to the intestine (Gao et al., 2021).

6.4.3 The effects of viscosity

Starch digestibility of the food also depends on the apparent viscosity of the bolus. Fermented rye bran extrudates and their bolus samples were less viscous compared to native rye bran (RB-40) and endosperm rye flour (RB-0) extrudates in this work. Due to their low viscosity, the fermented rye bran extrudates hydrolysed more readily than the native rye bran extrudates. Furthermore, the fermented rye bran extrudates had a significantly lower (6.3 vs 4.4) pH due to fermentation-induced acidification compared to the native rye bran extrudates. Acid production during bran fermentation probably caused a depolymerisation of the starch macromolecules such as amylose and amylopectin, which reduced the swelling capacity of the starch granules. Therefore, the starch in the fermented rye bran went through mild acid hydrolysation due to the low pH and thus perhaps reduced its viscosity. It has earlier been shown that acid hydrolysis of starch reduced the viscosity of cassava extrudates, where low pH (4.0) extrudates had lower viscosity (300 vs 350 cP) than the high pH (6.5) extrudates (Sriburi & Hill, 2000).

The solubilisation of DF determines the apparent viscosity of the food digesta (Capuano, 2017). Cell wall material in the fermented rye bran in this work probably hydrolysed due to the acidification. The degradation of the cell wall material reduced the swelling capacity of the bran particles and therefore reduced viscosity. EPS fermentation of rye bran has been shown to increase soluble pentosans in fermented bran (Nikinmaa et al., 2017), which might result in low viscosity. It has earlier been shown that enzymic hydrolysis of arabinoxylan and cellulose reduced viscosity (Park et al., 2019). The low viscosity in turn influences the starch hydrolysis in fermented rye bran extrudates by increasing the diffusion of digestive medium and thus accelerating the enzymic breakdown of starch. The high viscosity of the food digesta hinder the access of digestive fluids to the macromolecules of bolus (Lentle & Janssen, 2008). It has also been shown that acid hydrolysis increases rapidly digestible starch due to either disruption of the granular structure or increasing the effective surface area for enzymic attack by producing larger numbers of small particles (Zhang et al., 2006).

Visual observation of the boluses in this work revealed that the boluses obtained from native rye bran added extrudates as well as extrudates without rye bran had a more cohesive and compact structure compared to the boluses obtained from fermented rye bran. Some extent of the starch-protein matrix probably remained intact in the native bran extrudates during mastication and therefore remained less hydrolysed, which is in line with the observations of earlier work (Johansson et al., 2018). The viscosity of the bolus samples in this work was significantly lower (16–62%) than the viscosity of ground extrudates. The hydrolysis of the starch already started when the extruded samples were exposed to salivary α-amylase during mastication. Therefore, the main reason for viscosity reduction in the bolus was the hydrolysis of the starch by salivary α-amylase. Gelatinised starch granules become weaker and lose their mechanical energy when they are exposed to salivary α-amylase (Evans et al., 1986), which accelerates their disintegration. Not only the surface of the starch granule but also the whole starch chain is attacked by the α-amylase, which in this work caused a significant drop in the viscosity of the overall bolus. The disintegration of the fermented rye bran extrudates probably accelerated due to their crispy texture, constant stirring as well as the α-amylase activity. A higher number of small particles created more contact points when exposed to α-amylase. As a result, the boluses obtained from fermented rye bran extrudates were more hydrolysed and had resulted in a less viscous slurry compared to native rye bran bolus.

6.5 Limitations of the study

The extruded products examined in this work were prepared in various projects with varying aims, which resulted in some heterogeneity in the used materials and applied methods. The extruded products in Publications 1–4 were produced with varying amounts of rye bran with different particle sizes. In some cases, when the composition (bran-flour ratio) was very similar, e.g., when 10 or 15% rye bran was added to rye flour, it was difficult to compare the results to say where the textural differences were actually coming from. Moreover, the extrusion-processing conditions were different, which also made it difficult to compare the result obtained from different sub studies. Texture analysis of the extruded samples in Publication 1–2 was performed after equilibrated at a relative humidity of 43% in order to avoid moisture content related variation during analysis. On the other hand, extruded samples prepared in Publication 3–4 was not equilibrated because we had measured *in vivo* mastication and sensory analysis of those samples and thus we wanted to avoid any unpleasant sensory changes. Therefore, the extrudate properties obtained in the different publications were not absolutely comparable due to some methodological differences.

There were no differences in the mastication properties between puffs with or without rye bran. This indicates that subtle structural differences observed instrumentally between the products could not be detected due to the inter-subject variation. However, when the mastication was studied for one participant separately rather than taking the average values of all participants, the differences between the

products were significant. After Publication 3, we thought that a test subject size of more than fifteen would be sufficient to provide data with reasonable deviation and increased the subject size to twenty-six in Publication 4. However, no differences in mastication properties were seen even when 40% rye bran was added to the puffs and 26 individual participants masticated the extruded samples. The inter-individual differences in chewing behaviour requires to involve significantly higher amount of participants in the mastication tests to reduce the deviation which is quite challenging or even not practical due to complexity of the EMG test set-up. Moreover, all the participants were young females so the result obtained from this work cannot be generalized for all people. However, this work still provides useful information about the mastication patterns of different crispy extrudates and their effect on the bolus particle size and in turn starch digestibility.

In this work, an *in vitro* starch digestibility analysis was only performed for ground extruded products. It would have been better to study the starch digestibility of the original bolus sample obtained from different subjects. Then it would become more realistic to compare both the *in vitro* and *in vivo* starch digestibility results, which would have helped to draw better conclusions. Moreover, the exact digestion conditions in the stomach phase were not created for starch digestibility and viscosity analysis. For example, pepsin was not added to the medium. However, the same protocol was used for all the extruded samples and thus the results were reproducible and well comparable.

6.6 Future prospects

This work showed that particle size reduction is an effective way to improve the applicability of rye bran in extruded products. However, more research is needed to optimize the extrusion process conditions together with the particle size reduction, which will help to develop high DF extrudates with desired structures and textures. Fermentation was also shown to be a feasible tool to improve the rye bran functionality in extruded products even at a high bran addition level. Therefore, a combination of two bran modification techniques, i.e. bran fermentation and particle size reduction, could increase the utilization of rye bran in extruded snacks as well as in other high-fibre foods.

Both the structural and textural properties of food dictate the mechanical disintegration in the mouth. To date, very few studies have been conducted to study the impact of processing and ingredient interactions on the structure, texture and in turn the mastication of high DF extruded foods. It is thus crucial to understand the varying cellular structure and textural properties of DF enriched extrudates in order to predict their impact on mastication, physiological responses and to maintain food intake regulation. A profound understanding of the structural properties of extrudates with added rye bran and their role in disintegration and digestion will allow the design of new rye-based products with nutritionally optimal structures. The outcome of this work will greatly enhance the potential use of rye bran in food products and in turn

will assist in reducing obesity and chronic diseases among the rapidly increasing population.

More research should be conducted to find the optimal range of rye bran particle size and addition levels for producing bran-enriched snack products with a targeted improvement of both structure and texture as well as low starch digestibility. The results may be applied in the food industry as part of the attempt to increase the content of DF in palatable extruded foods. The findings and outcome will benefit the food-processing industry by delivering profound knowledge in understanding the process-structure-physiological functionality relationship, which is very important when designing new healthy foods which are rich in DF.

7 Conclusions

The extrusion processing of coarse rye bran without the addition of starch was challenging. Due to the high DF content, rye bran extrudates were less expanded, dense, and were less crispy than extrudates made of endosperm rye flour. To overcome these challenges the particle size of the rye bran was reduced from 440 to 28 μm. The particle size reduction of the rye bran made it applicable in directly expanded extruded products. Finely milled rye bran significantly improved the structural and textural properties of the extrudates. In addition to the bran particle size, the screw speed during the extrusion process also had a great influence on the physical properties of the extrudates. High screw speeds resulted in expanded and less hard extrudates in all experiments regardless of the particle size.

The results of this work depict that finely milled rye bran could be used as a potential low-cost dietary fibre source in extruded products. This work also showed that it was possible to use up to 30% finely milled rye bran in expanded extrudates without compromising the structural and textural properties. The results suggest that the technological functionality of rye bran can be improved through fermentation with the exopolysaccharide producing *Weisella confusa* strain. Both the structural and physicochemical properties of rye bran were affected by fermentation. The fermentation of rye bran resulted in extrudates with an improved structure and texture, which was also reflected in their perceived sensory attributes.

Expanded and crispy puffs required less mastication effort and easily disintegrated into smaller particles during oral processing compared to dense and crunchy flakes. The addition of rye bran attributed to a hard and less crispy texture in both puffs and flakes. However, subtle structural differences between similar product types (puffs vs puffs or, flakes vs flakes) due to bran addition were not reflected in mastication. The mastication properties of the extruded products were different only when there were large structural differences and the products were of different types (puffs vs flakes). Large inter-individual variation between the subjects prevented the detection of subtle textural differences within the same product category regardless of bran addition.

It was demonstrated that the starch digestibility of the extruded products could be influenced by microstructural properties. Extrudates with 30% fine rye bran had the crispiest structure but had lower starch digestibility compared to 15% fine bran extrudates, perhaps due to their high cell wall thickness. This work showed that the addition of 10% rye bran resulted in a higher number small particles in the bolus. The increased number of small particles led to an increase in the starch hydrolysis rate in all rye bran added products either puffs or flakes. Therefore, a small increase in the TDF content of extruded products due to bran addition may not always reduce the rate of starch digestibility and the effect may actually be opposite. Interestingly, the starch digestibility of fermented rye bran extrudates was higher compared to native rye bran extrudates, even though the total available starch was lower in the fermented rye bran. The structure-texture interplay in fermented rye bran extrudates overrode the difference in the total available starch by resulting in a more fragile and easier to digest structure. Fermentation induced changes in the bran material also reduced the viscosity, which might have an influence on increasing the rate of starch hydrolysis. Therefore, extrudates with similar bran compositions (native vs fermented rye bran, 40%) may be digested at different rates depending on their structural attributes.

Fermented rye bran in the extrudates increased the DF content and produced a palatable texture, which is considered the most important quality attribute in the snack food category. The higher starch digestibly of fermented rye bran might be disadvantageous from a nutritional point of view. However, a portion of 30 g of fermented rye bran extrudates will provide 6.6 g of DF and 16.2 g starch. As a result, the glycaemic load of a portion of fermented rye bran extrudates will remain reasonably low but will efficiently increase the DF intake. Therefore, fermented rye bran extrudates could be a potential healthy alternative to the snack product category due to their palatable texture and high DF content.

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PUBLICATION I

Influence of particle size reduction on structural and mechanical properties of extruded rye bran

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ORIGINAL PAPER

Influence of Particle Size Reduction on Structural and Mechanical Properties of Extruded Rye Bran

Syed Ariful Alam · Jenni Järvinen · Satu Kirjoranta · Kirsi Jouppila · Kaisa Poutanen · Nesli Sozer

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Abstract Rye bran is a high-fibre ingredient also containing starch and protein. The aim of this work was to investigate the effects of extrusion processing and bran particle size on the structural and mechanical properties of extruded rye bran. Native rye bran particle size of 750–1,250 μm was milled to produce feed material with three different average particle sizes (coarse, 440 μm; medium, 143 μm; fine, 28 μm). A co-rotating twin screw extruder was used for extrusion with various processing parameters. Extrusion processing did not have a significant ($P<0.05$) effect on soluble dietary fibre (SDF) content but the amount of insoluble dietary fibre (IDF) increased by 7.1–9.5 % in medium- and 11.3–12.3 % in fine-particle-sized rye bran extrudates as compared to the raw material prior to extrusion. Decreasing the particle size of rye bran to 28 μm resulted in lower amounts of IDF and total dietary fibre, but a higher amount of SDF after extrusion compared to coarse-particle-sized rye bran. Decreasing the particle size of rye bran to 28 μm gave more expanded (179–223 %), less hard (145–336 N), more crispy ($2.7–7.2\times10^{-4}$) and porous (79.2–83.9 %) extrudates compared to the coarse-particle-sized rye bran extrudates, which were less expanded (151–176 %), harder (210–433 N), less crispy ($0.5–2.8\times10^{-4}$) and less porous (64.4–65.1 %). Reduction of the particle size of rye bran significantly ($P<0.05$) increased the crispiness compared to the extrudates made of coarse-particle-sized rye bran. The results demonstrated that the structural and mechanical properties of rye bran extrudates can be improved without starch addition by reducing the particle size of bran.

Keywords Crispiness · Dietary fibre · Extrusion · Microstructure · Micronisation of particle · Texture · X-ray microtomography

S. A. Alam · J. Järvinen · K. Poutanen · N. Sozer (✉)
VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, 02044 Espoo, Finland
e-mail: nesli.sozeraykal@vtt.fi

S. A. Alam (✉) · S. Kirjoranta · K. Jouppila
Department of Food and Environmental Sciences, University of Helsinki, Agnes Sjöbergin Katu 2, P.O. Box 66, 00014 Helsinki, Finland
e-mail: ariful.alam@helsinki.fi

S. A. Alam
e-mail: ariful.alam@vtt.fi

Introduction

The consumer demand for palatable dietary fibre (DF)-enriched food products is currently growing. Several studies have shown that consumption of DF reduces the risk of obesity, cardiovascular disease, cancer and diabetes (Buttriss and Stokes 2008; Dahm et al. 2010). Despite these health benefits, the daily intake of DF in most developed countries still remains below the recommended level of 25–35 g/day. Snack foods have become a part of the prevailing lifestyle especially in the Western countries, and are generally made using refined flour- or starch-based products (e.g. corn, wheat, rice, oats and potatoes) lacking nutritional elements such as dietary fibre (Brennan et al. 2013). Bran is the outer part of the cereal grain, which contains a substantial amount of DF. Brans are separated in the milling process and are an important by-product of the cereal industry, mainly being used as animal feed (Kamal-Eldin et al. 2009). In rye grain, dietary fibre and phytochemicals are mainly concentrated in the bran fraction (Liukkonen et al. 2003; Kamal-Eldin et al. 2009). Rye bran contains 39–48 % DF including arabinoxylan (20–25 %) and ß-glucan (3.5–5.3 %), 13–28 % starch and 14–18 % protein (Kamal-Eldin et al. 2009; Karppinen et al. 2001, 2003). Hence, utilisation of rye bran as a raw material in snack food

formulations would give rise to fibre-enriched and healthy food products.

A variety of fibre-enriched snack foods can be prepared by extrusion (Lue et al. 1991; Sozer and Poutanen 2013). Extrusion processing is a high-temperature short-time process mainly used to produce puffed snacks directly by modification and texturization of the functional properties of food ingredients (Singh et al. 2007; Sozer and Poutanen 2013). The properties of extruded products depend strongly on the raw materials and processing conditions, in which high DF products exhibit poor macro- and microstructural and mechanical properties (Sozer and Poutanen 2013). A higher incorporation (10–30 %) of sugar beet fibre and (10–40 %) of soy fibre reduced expansion and increased both hardness and density along with a reduction in crispiness of corn flour-based extruded products (Lue et al. 1991; Jin et al. 1995). When a mixture of wheat flour and wheat bran (12.6–24.4 % DF) was extruded, more expanded extrudates were obtained using high screw speed and temperature with low moisture content, but increasing the bran concentration reduced the volumetric expansion under the same processing conditions (Robin et al. 2011b). Chassagne-Berces et al. (2011) also reported decrease in extrudate expansion by increasing the amount (10–20 %) of oat and wheat bran in wheat and whole wheat flour extrudates. Addition of bran to the extruded mass interferes with the continuity of food matrix, which affects both the structural and mechanical properties (Lue et al. 1991; Sozer and Poutanen 2013).

Feed material with high amounts of insoluble fibre source such as cereal bran could affect structural properties by rupturing the air cell wall of the extruded products, thus preventing the full expansion of air cells. Increasing the insoluble fibre content in extruded solid foam matrices increased the air cell wall thickness and decreased the cell sizes (Guan et al. 2004). This might be the reason for decreased expansion and crispiness and increased hardness and density with increased amounts of bran in the extruded feed material (Lue et al. 1990; Moore et al. 1990; Mendonça et al. 2000). Due to the adverse effect of cereal bran addition on the structural properties of the extrudates, typically 10–30 % of bran has been used previously (Sozer and Poutanen 2013). Many researchers (Lue et al. 1991; Desrumaux et al. 1998; Zhang and Hoseney 1998; Mathew et al. 1999; Al-Rabadi et al. 2011) reported that more expanded, less hard and crispier extrudates could be obtained by decreasing the particle size of the feed material (e.g. flour, grit and bran). Particle size of the feed material plays an important role in a product's physical and mechanical properties, which may vary considerably depending on the feed material type and the composition of the feed formulation, such as fibre to flour ratio (Guy 1994; Garber et al. 1997; Desrumaux et al. 1998; Mathew et al. 1999; Onwulata and Konstance 2006; Robin et al. 2011a; Alam 2012). However, the effect of particle size on the microstructural and mechanical properties of high-fibre extrudates has been reported in only a limited number of studies (e.g. Lue et al. 1990, 1991, Guan et al. 2004; Al-Rabadi et al. 2011; Robin et al. 2011a, b; Alam 2012). For example, Robin et al. (2011a) did not observe any significant increase in a products' expansion with reduced particle size of wheat bran in extrudates based on wheat flour. However, the average particle size used in their study was 317 μm for coarse and 224 μm for fine materials. We believe that a particle size reduction of only 30 % of the coarse material will not be sufficient to result in any perceivable structural and mechanical effects at 12 and 24 % bran addition.

The aim of this study was to investigate the effects of particle size reduction on the structural and mechanical properties of rye bran extrudates processed under various extrusion conditions without any flour or starch addition. This study also aimed to demonstrate how extrusion processing of rye bran (with various particle sizes) affects the soluble and insoluble fibre fraction.

Materials and Methods

Feed Material Preparation

Native rye bran (R4000) obtained from Fazer Mill and Mixes (Lahti, Finland) was milled to three different particle sizes of coarse, medium and fine (Table 1). Coarse and medium raw material were prepared at VTT Technical Research Centre of Finland (Espoo, Finland) by milling native rye bran in an Alpine fine impact mill at 17,800 rpm (100 UPZ, Hosokawa Alpine, Augsburg, Germany) twice with 1 and 0.3 mm sieves, respectively. Fine rye bran was milled using a G-55 Turborotor Mill (Görgens Mahltechnik GhmH, Dormagen, Germany) using 60 Hz motor cycles and an average feed rate of 30 kg/h.

Particle Size Analyses

Particle size distribution of the milled raw materials was measured with a laser diffraction particle size analyser (LDPSA; Beckman Coulter LS 230, Coulter Corporation, Miami, FL, USA) using the wet module. Particles were measured with a range of 0.04–2,000 μm. Milli-Q water was used as background solution. Particle size distributions were expressed in volume units and the measurements were made in duplicate. The particle size analysis using LDPSA for coarse-particle-sized bran was not complete due to the presence of particles larger than the range of LDPSA. Therefore, particle size distribution was also determined by sieving (Table 2). A rye bran sample of 100 g was sieved for 10 min using a laboratory sieve set (Buhler RP-MUL-300 Brabender, Duisburgh, Germany). Analyses were made in duplicate, and

Table 1 Composition of coarse-, medium- and fine-particle-sized bran

Component (%, dry weight basis)	Coarse	Medium	Fine
Protein	13.8±0.0 x	13.8±0.0 x	13.1±0.1 y
Fat	1.6±0.0 x	2.0±0.0 y	1.9±0.0 y
Starch	38.4±0.4 x	38.7±0.1 x	44.3±0.2 y
Total dietary fibre (TDF)	30.5±0.8 x	29.2±0.8 x	26.7±0.7 y
Insoluble dietary fibre (IDF)	25.7±0.4 x	24.1±0.9 y	21.2±0.7 z
Soluble dietary fibre (SDF)	4.8±0.4 x	5.1±0.1 xy	5.5±0.1 y
Ash	3.7±0.0 x	3.8±0.0 x	2.9±0.1 y

Values followed by the same letters (x–z) in the same row were not significantly different ($P<0.05$)

results were reported as averages of two measurements. The median particle size of the native rye bran was 750–1,250 μm as analysed by the sieving method.

Preliminary Study

The effects of extrusion processing conditions on macrostructure of rye bran extrudates and bran modification were studied in the preliminary studies in order to understand the basis of rye bran extrusion. A total of 36 samples were produced with coarse, medium and fine rye bran using different extrusion process parameters such as screw speed (300 and 500 rpm), temperature (40, 70, 75, 75, 90, 110, 110 and 40, 70, 75, 90, 95, 130, 130 °C in sections 1–6 and in the die), feed moisture (17 or 19 %) and hydration regimen (in barrel–water feed (IB) and preconditioning (PC)). The macrostructural properties of the extrudates were found to be strongly dependent on extrusion process parameters. The most important processing parameters were screw speed, specific mechanical energy (SME), barrel temperature profile and particle size of the feed material. In general, a combination of high screw speed and small bran particle size resulted in extrudates with highest expansion and lowest piece density. Feed moisture level did not affect the expansion ratio significantly, although a small decrease in expansion rates was observed as the feed moisture increased from 17 to 19 %. Neither hydration regimen nor barrel temperature profile influenced the expansion properties of rye bran extrudates.

The effects of processing parameters on DF content were studied only with coarse particle size at a feed moisture level of 17 % in order to provide an overview of the effect of extrusion on rye bran modification. Extrusion did not significantly affect the insoluble dietary fibre (IDF) or total dietary fibre (TDF) content of the coarse rye bran in any of the processing conditions used in this study, whereas the soluble dietary fibre (SDF) content was significantly increased in more intensive extrusion processing conditions. The hydration regimen had a significant effect on IDF and TDF contents. In preconditioned samples, IDF was decreased leading to a decrease in TDF. Other processing variables did not influence the DF components of extruded rye bran.

On the basis of the preliminary study, four samples of each particle size were selected for the final experiment. Since the microstructural properties of extrudates were significantly influenced by screw speed, both 300 and 500 rpm screw speeds were studied in the final experiments. Hydration regimens (both IB and PC) were also studied as these were the only parameters influencing DF contents. Feed moisture (17 %) and temperature profiles (40, 70, 75, 90, 95, 130 and 130 °C) were selected for further studies.

Extrusion Processing

Extrusion processing was performed using a co-rotating twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Dreieich, Germany). The length (L) and diameter (D) of the screw were 672 and 24 mm, respectively, and thus the L:D ratio was 28:1. The extruder barrel consisted of seven sections, among which the first one (section 0) included the gate for solid feed without temperature control; six had individual temperature control. Each section was 96 mm long. The diameter of the circular die was 5 mm (Fig. 1). A volumetric co-rotating twin-screw ($D=20$ mm, $L/D=10$) feeder (Brabender, Duisburg, Germany) was used for the solid feed. A full factorial experimental design was used for all extrusion trials (Table 3). The feed rate of 67 g/min was kept constant during the experiments. Screw speeds used in the extrusion experiments were 300 and 500 rpm. Two temperature profiles were used: low temperature profile (40, 70, 75, 75, 90, 110 and 110 °C) and high temperature profile (40, 70, 75, 90, 95, 130 and 130 °C) in sections 1–6 and in the die. The feed moisture was 17 or 19 %. With 19 % feed moisture, the water was fed into the extruder barrel immediately after the dry material feed. The injection gate for liquid feed was in section 1 and was controlled by a peristaltic pump (Watson Marlow 505S, Wilmington, MA, USA). This hydration regimen is referred to as in IB. With 17 % moisture, two

Table 2 Median particle size and particle size distribution of coarse-, medium- and fine particle-sized rye bran determined using a laser diffraction particle size analyser (LDPSA)

Bran	Median particle size (μm)	<1 μm (%)	<10 μm (%)	< 100 μm (%)	< 1000 μm (%)
Coarse	440±1.3 a	0.0±0.0 a	3.8±0.1 a	23.5±0.2 a	81.8±0.3 a
Medium	143±1.3 b	0.0±0.0 a	5.8±0.1 b	40.3±0.3 b	99.9±0.0 b
Fine	28±0.1 c	0.0±0.0 a	16.3±0.1 c	83.6±0.3 c	100.0±0.0 b

Values followed by the same letters (a–c) in the same column were not significantly different ($P<0.05$)

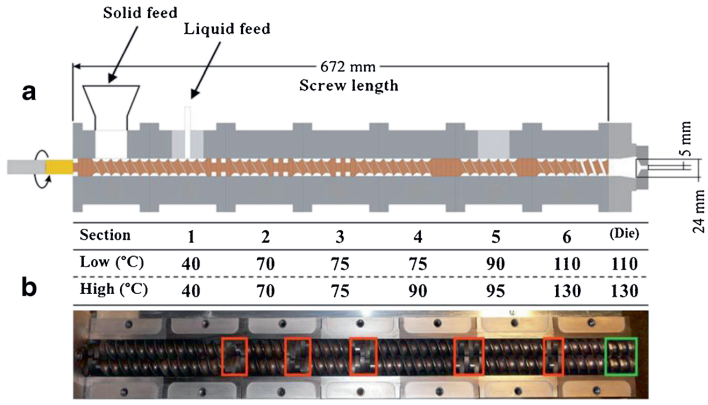

Fig. 1 **a** Extruder design and barrel temperature profile in *sections 1–6* and the die (adapted and modified from De Pilli et al. (2011)). **b** Configuration of twin screws inside the barrel. The kneading elements between conveying screws are highlighted in *red*. The pressurising elements also known as extrusion screws are marked in *green*

water addition regimens were used: IB and PC. PC was carried out by adjusting the moisture content of the bran before extrusion. The bran was mixed in a spiral mixer (Diosna SP 12 F/E, Dierks & Söhne, Osnabrück, Germany) and the required amount of water was added gradually with continuous high-speed mixing. A cover was used to prevent moisture loss during mixing. After completion of water addition, mixing was continued for another 10 min and the bran was hydrated overnight at 4 °C in sealed plastic bags.

The values of torque, die pressure and die temperature were monitored and recorded during the extrusion. SME is an important process parameter used to describe processing conditions. SME (Hu et al. 1993) was calculated with Eq. 1:

$$\text{SME}\ \left(\text{kW h kg}^{-1}\right) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q}. \quad (1)$$

Where ω is the screw speed (in rotations per minute, rpm), ω_r is the maximum screw speed of the extruder used (1,100 rpm), τ is the torque (in percent), Z_r is the maximum power capacity of the extruder (16 kW) and Q is the feed rate (in kilogram per hour).

Chemical Analyses

Analyses of the chemical composition of raw materials and extruded samples were made as follows: total protein content by American Association of Cereal Chemists (AACC) method no. 46-11A (AACC 2003), fat by Association of Official Analytical Chemists (AOAC) method no. 922.06 (AOAC 2000), total starch by AACC method no. 76.13 (AACC 1999), total dietary fibre by AOAC method no. 985.29 (AOAC 1990), insoluble and soluble dietary fibre by AOAC method no. 991.43 (AOAC 1992), ash gravimetrically by burning at 550 °C in a muffle furnace, and moisture content by drying the samples in an oven at 105 °C for 3 h. Total protein, fat and ash were analysed as duplicates, whereas total starch and all fibre analyses were performed as quadruplicates.

Macrostructural Analyses

Extruded samples (20 replicates) of each extrusion experiment were collected, dried at 105 °C for 20 min and cooled to room temperature. Length and diameter in three different points of each sample were measured using a Vernier calliper and the average diameters of the samples were obtained.

Expansion rate was calculated with Eq. 2:

$$\text{expansion rate}\ (\%) = \frac{D_e}{D_d} \times 100\% \quad (2)$$

Table 3 Experimental design for rye bran extrusion

Sample	Particle size (μm)	Hydration regimen (IB/PC)	Screw speed (rpm)
$\text{Coarse}_{\text{IB 300}}$	440	IB	300
$\text{Coarse}_{\text{IB 500}}$	440	IB	500
$\text{Coarse}_{\text{PC 300}}$	440	PC	300
$\text{Coarse}_{\text{PC 500}}$	440	PC	500
$\text{Medium}_{\text{IB 300}}$	143	IB	300
$\text{Medium}_{\text{IB 500}}$	143	IB	500
$\text{Medium}_{\text{PC 300}}$	143	PC	300
$\text{Medium}_{\text{PC 500}}$	143	PC	500
$\text{Fine}_{\text{IB 300}}$	28	IB	300
$\text{Fine}_{\text{IB 500}}$	28	IB	500
$\text{Fine}_{\text{PC 300}}$	28	PC	300
$\text{Fine}_{\text{PC 500}}$	28	PC	500

Extrudates made of coarse-, medium- and fine-particle-sized rye bran processed with 17 % feed moisture in two different hydration regimens (in barrel–water feed (IB), preconditioning (PC)) at two different screw speeds of 300 and 500 rpm at 130 °C

where D_e is the average diameter measured at three different points of the extrudate sample (in millimeter) and D_d is the diameter of the die (5 mm).

Specific length was calculated with Eq. 3:

$$\text{specific length}\left(\text{m kg}^{-1}\right) = \frac{L_e}{m_e} \tag{3}$$

where L_e is the length of the extrudate sample (in meter) and m_e is the mass of the sample (in kilogram). Piece density was calculated with Eq. 4:

$$\text{piece density}\left(\text{kg m}^{-3}\right) = \frac{4 \times m_e}{\pi \times (D_e)^2 \times L_e} \tag{4}$$

where m_e is the mass of the sample (in kilogram), D_e is the average diameter measured at three different points of the extrudate sample (in meter) and L_e is the length of the extrudate sample (in meter).

Microstructural Analyses

Samples for X-ray microtomography (XMT) were made by cutting 10 mm pieces (radially) from the extrudate samples with an electric saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan). Samples were scanned using a desktop XMT system (Model 1172, SkyScan, Aartselaar, Belgium) consisting of an X-ray tube, an X-ray detector and a charge-coupled devices (CCD) camera. The X-ray tube was operated at a voltage of 40 kV/250 μA to obtain optimum contrast between void (air cells) and matter (cell walls) according to a modified method (Sozer et al. 2011a, b). A 12-bit cooled CCD camera (512×1,024 pixels) was used to collect the X-ray data. Samples were rotated by a total of 180° during the scanning process with a pixel size of 11.65 μm to obtain optimum resolution, resulting in a total scanning time of 18 min. The initial X-ray radiographs or raw images were obtained at every 0.7° of rotation. Samples were scanned in triplicate. After scanning, radiographs were loaded into NRecon reconstruction software (v. 1.6.6). The software combines the images graphically into a 3-D object from which 2-D cross-sectional images can be taken. Before the reconstruction, the CS rotation feature was used to rotate the sample cross-sections, making them parallel to the view window. Beam hardening correction was set to 40 % in order to reduce the number of artefacts. Cell walls of the solid matrix appear grey, whereas air cells appear black. The reconstructed 2-D slices were then loaded into CTAn software (v. 1.12, Skyscan, Belgium) to obtain the parameters of porosity, cell wall thickness (t), cell diameter (D) and fragmentation index (i.e. degree of cell connectedness). In addition, the thickness to diameter (t/D) ratio was calculated from the data.

Mechanical Properties Analyses

Mechanical properties of extrudates were analysed by the uniaxial compression test using a texture analyser (Texture Analyser TA-HDi, HD3071, Stable Micro Systems, UK) equipped with a 250 kg load cell and a cylindrical 36 mm aluminium probe. Samples of 10 mm length were cut as described above and equilibrated at a relative humidity of 43 % at 21 °C. The samples were deformed at 70 % strain with a test speed of 1 mm/s. The force–deformation (f–d) curve was obtained to assess the mechanical characteristics of the extrudate samples. Each measurement was performed with 20 replicates. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain values of actual and smoothed curve length, area under the f–d curve, number of peaks and hardness (F_{max}). The number of peaks represents the number of cell wall ruptures during compression, and F_{max} is the maximum force needed to initiate cell wall crack. Different approaches were used to describe the hardness and crispiness of extrudates such as crushing force, crispiness work and crispiness index.

Crushing force was calculated with Eq. 5:

$$\text{crushing force}(N) = \frac{A}{l} \tag{5}$$

where A is the area under the f–d curve (Newton millimeter) and l is the distance of compression (in millimeter).

Crispiness work (C_w) was calculated with Eq. 6 (Van Hecke et al. 1998).

$$C_w(N\ \text{mm}) = \frac{A}{N} \tag{6}$$

where A is the area under the f–d curve (Newton millimeter) and N is the number of peaks.

Crispiness index (C_i) was calculated with Eq. 7 (Heidenreich et al. 2004).

$$C_i = \frac{L_N}{A \times F_{\text{mean}}} \tag{7}$$

where L_N is the normalised curve length (length of actual curve/F_{max}), A is the area under the f–d curve (Newton millimeter) and F_{mean} is the sum of the actual force values in the data file divided by the number of data points (N).

Statistical Analyses

Statistical significance was assessed by one-way ANOVA (LSD) using a significance level of 0.05. Correlations between different variables were calculated using two-tailed Pearson bivariate correlation with significance levels of 0.01 and 0.05.

Statistical analyses were made using SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA) software.

Results and Discussion

Dietary Fibre Content

The IDF and TDF contents of rye bran were decreased by milling (lowering the particle size from 440 to 28 μm), whereas the SDF content increased slightly. IDF and TDF contents of fine raw material were significantly lower than the coarse and medium materials (Table 1). The SDF content of fine rye bran was significantly higher than that of coarse bran. Coarse and medium rye bran had a significant difference only in IDF content, but both had equal amounts of TDF and SDF. Similar findings were reported by Zhu et al. (2010), who found that ultrafine milling of wheat bran to a final average particle size of 0.344 μm decreased IDF and TDF contents, but increased SDF content. The decrease of TDF content is caused by degradation of insoluble components (hemicellulose, cellulose and lignin) into smaller molecular weight compounds. On the other hand, decrease of IDF and increase of SDF were due to the redistribution of the fibre components from insoluble to soluble fraction. Extrusion processing had no significant effect on the DF composition of coarse rye bran (Table 4). However, IDF and TDF contents of medium and fine rye bran increased during extrusion processing. IDF contents increased by 7.1–9.5 and 11.3–12.3 % for extruded medium and fine rye bran, respectively, compared to the raw material. The TDF contents of extruded medium and fine bran increased by 6.2–9.9 and 9.0–10.5 %, respectively.

Extrusion did not have a clear effect on IDF, SDF and TDF contents of extruded rye bran samples. Extrusion processing has been reported to have less impact on the TDF content as compared to other physical DF modification techniques such as boiling, autoclaving and steaming (Meuser 2008). Increase of TDF was primarily due to the increase in IDF. The increase in IDF could be attributed to formation of resistant starch (Gualberto et al. 1997). The increase in SDF is due to the transformation of some of the IDF to SDF (Vasanthan et al. 2002). In the studies of Vasanthan et al. (2002), IDF, SDF and TDF contents of pearled grain barley flour increased as a result of extrusion. The processing conditions in their study were screw speed 50 rpm, varying moisture levels of 20–50 % and temperature of 90–140 °C. In earlier studies, Lue et al. (1991) reported that SDF content slightly increased in extruded corn meal and sugar beet fibre samples, but the differences were not statistically significant. In their experiments, extrusion conditions were mild (200/300 rpm, 25 % feed moisture and temperature of 120 °C). Esposito et al. (2005) also reported an increase in IDF of durum wheat bran after extrusion at 400 rpm and 140 °C. Gajula et al. (2008) and Zhang et al. (2011) reported conflicting results regarding IDF, TDF and SDF contents. In their study, IDF and TDF of wheat bran decreased during extrusion processing, whereas SDF content increased. The processing conditions in their studies were less severe compared to those of the current study (300/500 rpm, 17/19 % feed moisture and temperature of 110/130 °C). Gajula et al. (2008) used a screw speed of 200 rpm, feed moisture of 30 % and maximum barrel temperature of 40 °C, whereas in the study by Zhang et al. (2011), the corresponding conditions were 200 rpm, 45 % and 150 °C. Other studies on other DF sources (wheat bran and orange pulp) reported an increasing effect of extrusion processing on SDF content and a decreasing effect on TDF content (Wang et al. 1993; Larrea et al. 2005). The impact of extrusion processing on various DF components is related to several factors such as SME, screw speed, temperature, source of DF and particle size (Robin et al. 2011b).

Table 4 Insoluble (IDF), soluble (SDF) and total dietary fibre (TDF) contents of raw and extruded coarse-, medium- and fine-particle sized rye bran

Sample	Coarse	Medium	Fine
	IDF (%, dry weight basis)		
Raw	25.7±0.5 abcd, x	24.1±1.0 a, y	21.2±0.7 a, z
Extruded			
IB 300	26.9±1.1 aef, x	25.8±1.5 bcd, xy	23.8±1.1 b, y
IB 500	26.7±1.3 bge, x	26.1±0.9 bef, x	23.6±0.5 bc, y
PC 300	24.6±1.7 ch, x	26.4±0.1 ce, y	22.1±0.2 ad, z
PC 500	25.2±0.9 dfgh, x	25.3±0.6 adf, x	22.5±0.6 cd, y
	SDF (%, dry weight basis)		
Raw	4.8±0.5 abc, x	5.1±0.1 ab, xy	5.5±0.1 a, y
Extruded			
IB 300	5.1±0.3 adef, x	5.4±0.3 acde, x	5.4±0.6 a, x
IB 500	5.4±0.1 dgh, x	5.3±0.2 bc, x	5.9±0.1 a, y
PC 300	5.3±0.4 begi, x	5.7±0.1 df, x	5.8±0.1 a, x
PC 500	5.3±0.2 cfhi, x	5.7±0.3 ef, x	5.8±0.0 a, x
	TDF (%, dry weight basis)		
Raw	30.5±0.9 abcd, x	29.2±1.0 a, x	26.7±0.7 a, y
Extruded			
IB 300	32.0±0.9 aef, x	31.2±1.7 bcd, x	29.1±0.7 b, y
IB 500	32.2±1.2 be, x	31.3±0.8 bef, x	29.5±0.5 b, y
PC 300	29.9±1.4 cg, x	32.1±0.1 ce, y	27.9±0.3 ac, z
PC 500	30.4±0.9 dfg, x	31.0±0.8 df, x	28.3±0.6 c, y

Extrudates made of coarse-, medium- and fine-particle sized rye bran processed with 17 % feed moisture in two different hydration regimens (in barrel–water feed (IB), preconditioning (PC)) at two different screw speeds of 300 and 500 rpm at 130 °C

Values followed by the same letters (a–i) in the same column in each fibre group (IDF, TDF and SDF) were not significantly different ($P<0.05$). Values followed by the same letters (x–z) in the same row were not significantly different ($P<0.05$)

Extrusion did not significantly alter the SDF content except in the case of preconditioned medium-particle-sized rye bran, with which the amount of SDF increased (Table 4). The extrudates made of fine-particle-sized bran resulted in lower amounts of IDF and TDF and a higher amount of SDF compared to the extrudates made from coarse- and medium-particle-sized bran. The IDF, SDF and TDF contents of extruded samples varied in the ranges 22.1–26.9 %, 5.1–5.9 % and 27.9–32.2 %, respectively (Table 4). Processing parameters such as feed moisture, screw speed and SME had no significant effect on the DF contents of extruded bran samples.

Macrostructural Properties

Due to the high content of starch in rye bran, expanded products were produced in extrusion even without the addition of another starch source. Feed material had a starch content of 39–44 % of solids and a DF content of 26.7–30.5 % in the current study. In most studies, the amount of total starch content of the feed material is higher (55–79 %), whereas the fibre content is lower (12–30 %; Lue et al. 1991; Robin et al. 2011a, b; Saeleaw et al. 2012). The higher amount of starch and lower amount of fibre favoured expansion in the study by Lue et al. (1991), Robin et al. (2011a, b) and Saeleaw et al. (2012). Expansion rates of rye bran extrudates processed under different conditions are shown in Table 5. The highest expansion rates (223 and 228 %) were for fine rye bran extrudates processed with 17 % feed moisture (IB and PC) at 500 rpm, whereas the lowest expansion rates (141 and 150 %) were measured for medium rye bran processed with 17 % feed moisture (IB and PC) at 300 rpm.

Table 5 Macrostructural properties of rye bran extrudates

Sample	Expansion rate (%)	Specific length (m/kg)	Piece density (kg/m^3)
$Coarse_{IB\ 300}$	151±4 a	29±0.6 a	781±35 a
$Coarse_{IB\ 500}$	175±4 bcd	34±1.1 b	494±21 b
$Coarse_{PC\ 300}$	163±3 e	30±0.5 cd	629±19 c
$Coarse_{PC\ 500}$	176±3 bfg	35±0.4 efg	466±14 d
$Medium_{IB\ 300}$	150±4 a	30±1.0 chi	760±31 e
$Medium_{IB\ 500}$	175±4 cfh	35±0.7 ejk	475±18 d
$Medium_{PC\ 300}$	141±5 i	31±1.8 l	825±37 f
$Medium_{PC\ 500}$	165±3 e	35±1.4 fjm	531±21 g
$Fine_{IB\ 300}$	179±10 dgh	30±1.5 dhn	538±60 g
$Fine_{IB\ 500}$	228±11 j	34±1.3 b	292±32 h
$Fine_{PC\ 300}$	217±7 k	29±1.4 ain	368±20 i
$Fine_{PC\ 500}$	223±9 l	36±1.6 gkm	288±21 h

Extrudates made of coarse-, medium- and fine-particle-sized rye bran processed with 17 % feed moisture in two different hydration regimens (in barrel–water feed (IB), preconditioning (PC)) at two different screw speeds of 300 and 500 rpm at 130 °C

Values followed by the same letters (a–n) in the same column were not significantly different ($P<0.05$)

Reduction of particle size and increase in screw speed significantly increased the expansion rate of extrudates. Particle size reduction from 440 (coarse) to 143 μm (medium) had no significant influence on expansion rate, but further micronization to 28 μm had a pronounced effect on expansion. Similar results were reported by Robin et al. (2011b), who found that particle size reduction of wheat bran from 317 (coarse) to 224 μm (fine) did not significantly affect the expansion properties of wheat flour extrudates containing wheat bran. The particle reduction in Robin et al. (2011b) was only 30 % of the coarse (317 to 224 μm) wheat bran, whereas in this study particle size reduction was 94 % of the coarse (440 to 28 μm) rye bran. Alam (2012) showed that there is a critical bran concentration above which the effect of particle size reduction is significant. In that study, 10 % addition of defatted oat bran concentrate of three different particle sizes (32, 61 and 213 μm) to defatted oat endosperm flour (18 μm) did not give rise to any significant changes in the expansion properties.

These results are supported by the results of an earlier study by Lue et al. (1991), who found that decreasing the particle size of sugar beet fibre (from 2,000 to 74 μm) improved radial expansion of corn flour extrudates containing this DF source. Lue et al. (1990) suggested that decreased particle size of the sugar beet fibre favoured expansion due to the presence of more air cells compared to the coarse fibre. Particle size reduction of corn flour (or grits) has been reported to increase expansion in many studies (Garber et al. 1997; Desrumaux et al. 1998; Zhang and Hoseney 1998; Mathew et al. 1999; Onwulata and Konstance 2006). Increase of product expansion with particle size reduction was also reported by Al-Rabadi et al. (2011) in a study of milled barley. During extrusion, starch molecules entrap the fibre particles and form starch–fibre matrices. Therefore, fibre particle size has an important role in forming the cell structure. Lowering the particle size increased the possibility to obtain an expanded product by promoting foam expansion (Guan et al. 2004). The finer the fibre, the greater were the continuities in the starch–fibre matrices, thus reducing premature cell rupture during the extrusion. Larger particles have been suggested to restrict the development of air cells, disrupt the air cell walls and to cause air cells to collapse before optimal expansion takes place. Thus, materials with coarse particle resulted in less expanded products compared to fine materials. In addition, fine particle size may provide more nucleation sites and thus more air cells, resulting in better expansion (Lue et al. 1991). In the present study, increase in expansion rate by reduction of particle size to 28 μm could also be explained partly by the higher amount of starch in fine raw material. A lower amount of IDF in fine raw material could also be the reason for increased expansion.

A higher amount of IDF has been reported to reduce expansion rate since it causes premature rupture of air cells within the extrudate structure, reducing the air cell size and thus expansion (Yanniotis et al. 2007; Brennan et al. 2008).

Specific length of rye bran extrudates ranged between 29 and 35 m/kg (Table 5). Increase in screw speed significantly increased the specific length of extrudates. Higher screw speed probably decreased melt viscosity because of increased shear, resulting in a longer specific length. A similar result was reported by Jin et al. (1994) for corn flour and soy fibre extrudates. Particle size and hydration regimen did not have a significant effect on specific length.

Piece density of rye bran extrudates ranged between 288 and 825 kg/m^3 (Table 5). Coarse and medium rye bran had the highest density, whereas fine rye bran extrudates were least dense. Guan et al. (2004) stated that piece density is an indicator of a products' porosity and expansion. Decreased piece density with decreasing particle size was reported in their study when oat (360–420 μm), wood (250–350 μm) and cellulose (150–250) fibre was added to starch containing 70 % amylose. Increase in screw speed and SME significantly decreased piece density, which is in agreement with previous studies (Jin et al. 1994; Guha et al. 1997). Hydration regimen did not exhibit a clear trend with the piece density of extrudates.

Microstructural Properties

XMT image analysis was carried out for the extrudates processed at a screw speed of 500 rpm. Representative 2-D images of XMT analysis are shown in Fig. 2. Particle size reduction not only increased the expansion rate but also increased the diameter of air cells. The largest and most spherical air cells were observed in the centre of extrudates, whereas cell anisotropy was found close to the outer surface. From the image analysis data, the porosity increased from 64.4 to 83.9 % by reducing particle size from 440 to 28 μm (Table 6). Higher porosity of the fine particle size extrudates indicate that starch granules were less disrupted and tended to cause higher expansion compared to coarse and medium rye extrudates. Similar explanation was reported in the study by Singkhornart et al. (2014). However, no effect of particle size reduction was observed between coarse (440 μm) and medium (143 μm) rye bran extrudates. A good correlation was found between macro- and microstructural properties (Tables 5 and 6), where a positive correlation was found between expansion and porosity ($r=0.966$, $P<0.01$). Piece density was negatively correlated with both expansion ($r=-0.994$, $P<0.01$) and porosity ($r=-0.971$, $P<0.01$). This correlation indicates that the increase in expansion gives increased porosity and decreased

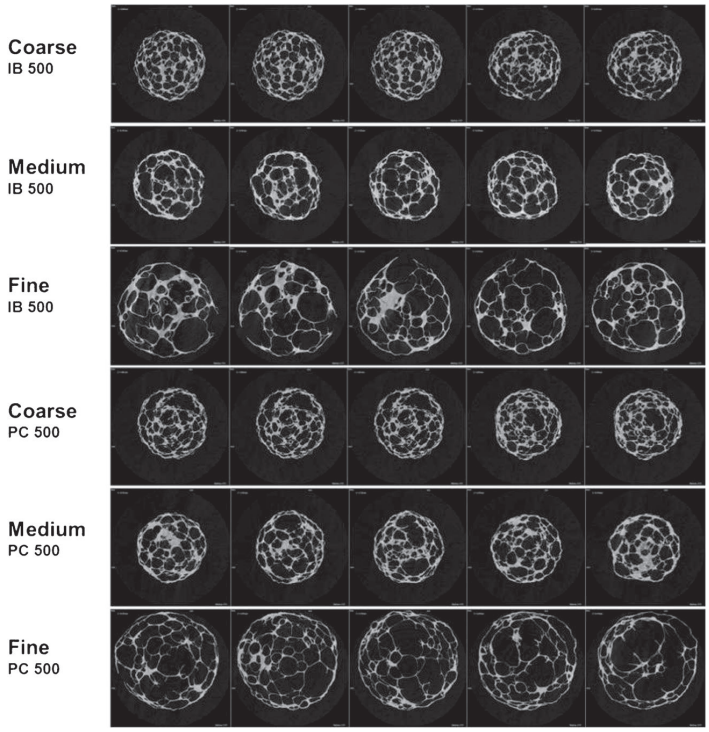

Fig. 2 2-D cross-section images of extrudates made of coarse-, medium- and fine-particle-sized rye bran processed with 17 % feed moisture in two different hydration regimens (*IB* in barrel–water feed, *PC* preconditioning) at a screw speed of 500 rpm at 130 °C

Table 6 Extrudate microstructural properties based on XMT image analysis

Sample	Porosity (%)	t (mm)	D (mm)	t/D	FI (1/mm)
$Coarse_{IB\ 500}$	64.4±0.4 abc	0.24±0.01 a	0.49±0.02 abc	0.50±0.03 abc	−4.5±0.7 abc
$Coarse_{PC\ 500}$	65.1±0.9 ade	0.24±0.01 a	0.51±0.04 ade	0.46±0.03 ade	−4.4±0.2 ade
$Medium_{IB\ 500}$	65.0±3.3 bdf	0.29±0.02 bc	0.62±0.07 bdf	0.47±0.08 bdf	−3.7±1.8 bdf
$Medium_{PC\ 500}$	64.4±1.7 cef	0.29±0.01 bd	0.63±0.11 cef	0.48±0.10 cef	−4.5±1.4 cef
$Fine_{IB\ 500}$	79.2±1.4 g	0.32±0.04 cd	1.17±0.03 g	0.28±0.02 g	−0.8±0.7 g
$Fine_{PC\ 500}$	83.9±2.3 h	0.20±0.01 e	1.10±0.14 g	0.18±0.03 g	−1.5±0.4 g

t Average cell wall thickness, D average cell diameter, FI fragmentation index

Extrudates made of coarse-, medium- and fine-particle-sized rye bran processed with 17 % feed moisture in two different hydration regimens (in barrel–water feed (IB), preconditioning (PC)) at a screw speed of 500 rpm at 130 °C

Values followed by the same letters (a–h) in the same column were not significantly different ($P<0.05$)

piece density and is in agreement with previous work reported by Robin et al. (2012) and Singkhornart et al. (2014).

Average cell diameter ranged between 0.49 and 1.17 mm and was significantly affected by reduction of particle size of rye bran down to 28 μm. Average cell diameter was highest for fine rye bran extrudates (Table 6). More small air cells were obtained with coarse- and medium-particle-sized extrudates (≈2,800–4,000) as compared to fine extrudates (≈1,000) due to early rupture of the precursor air cells (Robin et al. 2011b, 2012). The effect of particle size was more profound on D than on t, which affects the t/D ratio. Micronization of bran resulted in less thick cell walls and more expanded cells; the t/D ratio decreased from 0.5 to 0.18 (Table 6). The thin cell walls of whole wheat extrudates also gave expanded and less dense structure in the study by Singkhornart et al. (2014). Different hydration regimens had no influence on the microstructural parameters of coarse- and medium-particle-sized rye bran extrudates. However, preconditioning of fine-particle-sized bran increased the porosity by 6 % and decreased the t/D ratio almost by 50 %. The fragmentation index (FI) is a measure of the relative connectivity of the cell structure. Low FI values (i.e. higher negative values) are an indicator of more connected lattices, whereas a high value indicates a more disconnected structure (Hahn et al. 1992; Sozer et al. 2011b). FI of rye bran extrudates ranged between −0.8 and −4.5 l/mm indicating high connectivity of the pores (Table 6).

Mechanical Properties

Mechanical properties are important for expanded products since they can be used to predict sensory attributes of the products. Mechanical properties of rye bran extrudates were measured by the uniaxial compression test using a texture analyser. An f–d curve for a crispy product consists of a jagged curve in which there are many simultaneous fracture events.

Hardness indicates the maximum force needed to cause a cell wall rupture during compression. Hardness of coarse-, medium- and fine-particle-sized rye bran extrudates processed in various operating conditions is shown in Fig. 3a. Extrudates from fine rye bran were the least hard, ranging between 145 and 336 N, whereas coarse and medium particle size resulted in extrudates with hardness values from 210 to 460 N. Extrudates from fine particle size processed at higher screw speed had hardness values below 200 N, which has been stated to be a critical threshold for consumer acceptance (Lobato et al. 2011). Increase in screw speed significantly increased expansion and in turn decreased hardness of the products ($r=-0.790$, $P<0.01$). Alam (2012) showed that expansion and hardness of the extrudates made of defatted whole grain oats was significantly affected by the screw speed, which had the most significant negative effect on the hardness compared to other extrusion process parameters such as moisture content and feed rate. Hardness had a negative impact on product porosity ($r=-825$, $P<0.05$), which is in agreement with a study by Chanvrier et al. (2013). Hardness and crispiness index were negatively correlated ($r=-0.718$, $P<0.01$), indicating that extrudates with lower hardness values were more crispy. In addition to screw speed, particle size of rye bran was another significant factor affecting the textural properties of rye bran extrudates. Reducing the particle size of raw material from 440 to 28 μm resulted in extrudates with decreased hardness and increased crispiness. Garber et al. (1997) reported that decreasing the particle size of corn flour and grits from 1,622 to 50 μm increased the expansion and decreased the bulk density of the products, which finally dictated the decrease of hardness.

Crushing force (i.e. the average puncturing force) of extrudates varied from 35 to 108 N (Fig. 3b). Crushing force decreased with particle size ($r=0.631$, $P<0.05$). Increase in screw speed decreased crushing force, but the effect was not significant. Crispiness work varied from 2.95 to 16.6 N mm. The lowest crispiness work (2.95–4.16 N mm) was obtained with the fine-particle-sized bran. The higher the crispiness

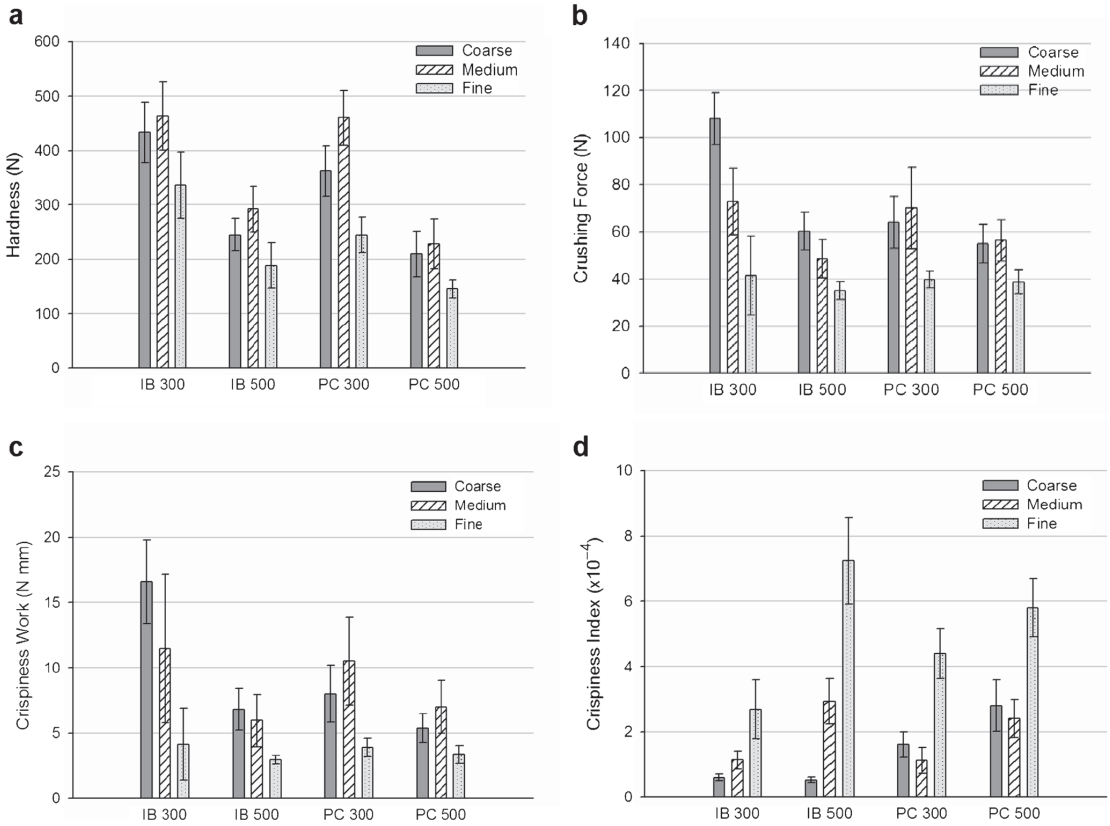

Fig. 3 **a** Hardness, **b** crushing force, **c** crispiness work and **d** crispiness index of the extrudates made of coarse-, medium- and fine-particle-sized rye bran processed with 17 % feed moisture in two different hydration regimens (*IB* in barrel–water feed, *PC* preconditioning) at two different screw speeds of 300 and 500 rpm at 130 °C

work, the more resistant and the harder is the product. Extrusion had similar effects on crispiness work (Fig. 3c) to those on crushing force, and the two results were in good agreement. The crispiness work obtained with fine-particle-sized bran (26.7 % TDF) was lower than that of the coarse-particle-sized bran (30.5 % TDF), which is in contrast to the studies

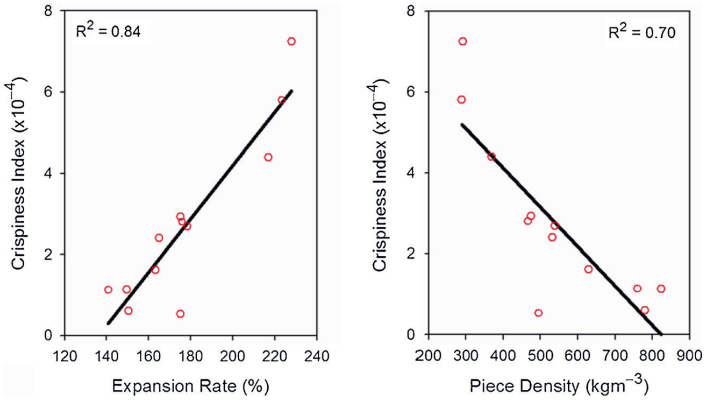

Fig. 4 Relationship between crispiness index, expansion rate and piece density

of Carvalho et al. (2010) and Alam (2012). On the other hand, in the extrusion study by Alam (2012), 10 % oat bran (particle size of 32, 61 and 213 μm) was added to oat flour (18 μm). In that study, the total fibre content of the feed material was 7 %, at which the concentration of fibre in the feed material was insufficient to reflect the impact of particle size reduction on structural and textural properties. Thus we hypothesise that the presence of less fibre in the feed material with smaller particle size might cause it to act as a filler within the starch–fibre matrix, which would result in a rigid and less crispy cell structure.

Crispiness index of the extrudates varied from 0.53×10^{-4} to 7.2×10^{-4}. Extrudates with fine-particle-sized bran had the highest crispiness indices with all treatments (Fig. 3d). This was reflected by the good correlation ($r=0.642$, $P<0.05$) between particle size and crispiness index. In most samples, higher screw speed also increased the crispiness index of extrudates significantly (Fig. 3d). Extrudates with higher expansion and lower piece density had increased crispiness index as shown in Fig. 4. Specific length of extrudates did not influence textural parameters, suggesting that radial expansion rate is a more significant parameter in predicting the textural attributes of extruded food products. Particle size reduction increased porosity and reduced cell connectedness (i.e. air cell structure was more open), leading to more expanded, less hard and more crispy products. Crispiness index showed a negative correlation with microstructural t/D ratio ($r=-0.876$, $P<0.05$) and a positive correlation with porosity ($r=0.882$, $P<0.05$) obtained from XMT image analysis. Good correlation between crispiness index and porosity suggests that crispiness of the product depends on the interior cell structure and cell wall morphology of the product (Chanvrier et al. 2013). Different particle sizes of rye bran possibly had a different cell structure and different cell wall morphology. Chanvrier et al. (2013) hypothesised that expanded cell structure and cell wall morphology affects the fracture mechanism, which controls the fracture events occurring in the cell wall during compression. Expanded extrudates with higher porosity had higher crispiness index values, whereas small air cells with thicker cell walls created foam structures with low crispiness index. The energy required to fracture large air cells surrounded by thin cell walls is lower than that required for small air cells with thick cell walls. Micronization of bran particles to 28 μm improved both the structural and mechanical properties of the rye bran extrudates.

Microstructural parameters such as fragmentation index, porosity and cell t/D ratio are related to textural parameters. The relationship between textural and microstructural parameters was also studied by Karkle et al. (2012), who reported that increase in average cell diameter increased the frequency of spatial ruptures (corresponding to the number of peaks in our study) and decreased crispiness work, indicating that extrudates with larger air cells were less hard and more crispy.

Conclusions

The effects of extrusion processing on rye bran were highly dependent on particle size of the raw material. The effects on dietary fibre profile were articulated for medium and fine rye bran compared to coarse rye bran. Extrusion processing did not have a significant effect on SDF content but TDF contents of medium and fine bran were increased by extrusion. Decreasing the particle size of the rye bran from 440 to 28 μm caused significant changes in the physical properties of the extrudates compared to the extrudates made of larger-particle (143 and 440 μm)-sized rye bran. The physical properties of rye bran extrudates were not only dependent on the particle size of the rye bran but also on extrusion process parameters such as screw speed. Screw speed had a great influence on product expansion and hardness. Regardless of particle size, high screw speed gave expanded and less hard extrudates in all extrusion experiments. Decreasing the particle size improved the crispiness of rye bran extrudates by increased expansion, air cell size and porosity with reduced hardness. Expansion properties of extruded products contribute to textural attributes, such as crispiness which is one of the most important consumer attributes for expanded snacks and cereals. Reduction of particle size led to a significantly higher crispiness index for all treatments regardless of screw speed and application of different hydration regimens. The general focus of this paper was to understand the effects of extrusion processing on rye bran by varying bran particle size with an emphasis on structure–texture–fibre effects. Future work will focus on starch–fibre interactions in model products.

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PUBLICATION II

Factors affecting structural properties and *in vitro* starch digestibility of extruded starchy foams containing bran

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Factors affecting structural properties and *in vitro* starch digestibility of extruded starchy foams containing bran

Syed Ariful Alam [a, b, *], Jenni Järvinen [a], Harri Kokkonen [c], Jukka Jurvelin [c], Kaisa Poutanen [a], Nesli Sozer [a, **]

[a] VTT Technical Research Centre of Finland, Tietotie 2, FI-02150, Espoo, Finland
[b] Department of Food and Environmental Sciences, Agnes Sjöbergin katu 2, P.O. Box 66, 00014, University of Helsinki, Helsinki, Finland
[c] Department of Applied Physics, University of Eastern Finland, P.O. Box 1627, 70210, Kuopio, Finland

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ABSTRACT

Rye bran of two different particle sizes (coarse: 440 μm and fine: 28 μm) were prepared by milling of commercial rye bran. Coarse and fine rye bran was added into a blend of rye endosperm flour and corn starch (70:30) to achieve two bran levels, 15 or 30%, to produce directly puffed extrudates. A co-rotating twin screw extruder was used with a screw speed of 500 rpm, barrel temperature profile: 40-70-75-90-95-110-110 °C and constant feed rate of 67 g/min. Feed moisture content of 17% was used either as in barrel-water feed or as preconditioning. Fine bran addition effectively improved macrostructural properties as compared to coarse bran through increasing expansion by 3.3–11.7% and piece density by 3.8–10.5%. Reduction of bran particle size significantly ($P < 0.05$) increased crispiness by 66.7–203.3%. Particle size reduction of bran had only minor influences on cell wall thickness, cell area and hydrolysis index of the extrudates. Extrudates made with 30% fine bran at in barrel-water feed provided the crispiest extrudates with lower *in vitro* hydrolysis index. The results demonstrated that the macrostructural and mechanical properties of extrudates containing rye bran can be improved by reducing bran particle size.

1. Introduction

Rye is a key source of dietary fibre (DF) and the second most common grain after wheat used in bread production in Europe; however the use of rye in extruded snack products is limited. Snack foods are often made of refined flour and starch (corn, wheat, rice, oats and potato), and thus these products tend to be low in DF, protein and other essential nutrients (Robin et al., 2012). There is an increasing consumer demand for healthy convenience and snack foods with high DF content. Several studies have shown that consumption of DF reduces the risk of obesity, cardiovascular disease, cancer and diabetes (Smith and Tucker, 2011; Hauner et al., 2012).

Rye bran, a by-product generated during rye flour processing, consists of 38–48% DF, 14–18% protein and 13–28% starch, and therefore could be utilized as a low-cost fibre and protein source in healthy snack formulations (Alam et al., 2014). High GI (glycaemic index) foods such as starch based extruded products have been reported to associate with increasing health risks related to obesity, diabetes and coronary heart disease (Livesey et al., 2008). Thus snack products with low GI and high DF are of interest for consumers and consequently food manufacturers. Processing method, feed material composition, microstructure and textural properties of food influence starch digestibility (Singh et al., 2013). It is thus important to understand how physical, textural and microstructural properties of starchy snack foods supplemented with DF affect the digestibility of starch.

The properties of extruded products depend strongly on the raw materials, level of incorporation and the source of the DF and finally on the processing conditions (Altan and Maskan, 2011). High fibre extrusion is challenging, resulting often in highly dense, less crispy and hard textures, which are not appreciated by consumers (Robin et al., 2012; Sozer and Poutanen, 2013). Cereal fractions rich in insoluble DF have poor gas-holding capacity and interfere with the expansion of air cells (Singh et al., 2007). Addition of bran in starch based products interferes with the continuity of starchy matrix.

* Corresponding author. VTT Technical Research Centre of Finland, Tietotie 2, FI-02150, Espoo, Finland.
** Corresponding author.
E-mail addresses: ariful.alam@vtt.fi (S.A. Alam), nesli.sozer@vtt.fi (N. Sozer).

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Abbreviations

A_{uc}	Area under the curve
C_i	Crispiness index
C_w	Crispiness work
D	Average cell diameter
DF	Dietary fibre
GI	Glycaemic index
HI	Starch hydrolysis index
IB	In barrel-water feed
PC	Preconditioning
SDF	Soluble dietary fibre
t	Average cell wall thickness
TDF	Total dietary fibre
WAI	Water absorption index
WSI	Water solubility index
XMT	X-ray microtomography

This affects both structural and mechanical properties thus reducing the overall quality (Sozer and Poutanen, 2013).

Due to the adverse effect of cereal bran on product quality, typically only 10–32% of bran has been added in previous studies as reviewed by Robin et al. (2012) and Sozer and Poutanen (2013). Several researchers reported that more expanded, less hard and crispier extrudates could be obtained by decreasing the particle size of the feed material such as fibre and wholegrain (Lue et al., 1991; Mathew et al., 1999). To date, a number of studies have been conducted to determine the effect of insoluble fibre addition and particle size reduction on the structural and textural properties of extruded products with different fibre sources such as sugar beet fibre (Lue et al., 1991), oat-fibre and cellulose (Guan et al., 2004), wheat bran (Robin et al., 2011a; Santala et al., 2014), oat bran (Sibakov et al., 2014) and rye bran (Alam et al., 2014).

In our previous study (Alam et al., 2014) a decrease in rye bran particle size from 440 to 28 μm improved overall quality of 100% rye bran extrudates. Particle size reduction of wheat bran (702 → 84 μm) improved expansion but did not affect textural attributes when 20% wheat bran was added to rye flour based extrudates (Santala et al., 2014). Robin et al. (2011a) and Sibakov et al. (2014) observed no significant increase in degree of expansion by adding 12–24% wheat bran (317 → 224 μm) and 10% oat bran (213 → 32 μm) into wheat and defatted oat flour based extrudates, respectively. Therefore, effect of particle size reduction on structural and textural properties needs to be further evaluated through macro- (expansion), and micro-structural properties such as porosity and cell wall thickness. To the best of our knowledge literature is missing the effect of bran particle size reduction on *in vitro* starch digestibility of high fibre starchy extrudates. The aim of the current work was to understand the factors (eg. hydration regimen, dietary fibre content and particle size) effecting the structural properties and *in vitro* starch digestibility of starch-based rye bran-enriched extrudates.

2. Materials and methods

2.1. Feed material preparation

Native rye bran obtained from Fazer Mill and Mixes (Lahti, Finland) was milled to two different particle sizes, coarse and fine. Coarse (D_{50} = 440 μm) and fine (D_{50} = 28 μm) rye bran were prepared at VTT Technical Research Centre of Finland (Espoo, Finland) by milling native rye bran using a published protocol used in our previous study (Alam et al., 2014). Rye endosperm flour (with a DF content of 7%, carbohydrate of 75% and protein content of 6% as detailed in the manufacturer specifications) was obtained from Helsinki Mills Ltd., Järvenpää, Finland. Waxy corn starch with 97% of amylopectin was from Roquette Ltd., France and was used to make starch-flour mixture by adding 30% of starch into 70% of rye flour using a spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany). This starch-flour mixture was used as extrusion feed together with coarse- or fine-particle sized rye bran so that the amount of bran was either 15% or 30% in the starch-flour-bran mixtures. Two different bran addition level categorises as low (15% bran: 82% starch and 8.2% DF) and high (30% bran: 74% starch and 12.6% DF) fibre feed material. Rye bran was mixed with starch-flour mixture prior to extrusion using the same spiral mixer to make the final blend of starch-flour-bran.

2.2. Particle size analyses

Particle size distribution of the milled raw materials was determined with a Laser Diffraction Particle Size Analyser (LDPSA) (Beckman Coulter LS 230, Coulter Corporation, Miami, USA) using the wet module. MilliQ-water was used as background solution. Particle size distributions were expressed in volume units and the measurements were made in duplicate.

2.3. Extrusion processing

A co-rotating twin-screw extruder (Poly Lab System, Thermo Prism PTW24, Thermo Haake, Dreieich, Germany) was used for extrusion trials using a method described by Alam et al. (2014). A full factorial experimental design was used for all extrusion trials (Table 1). The feed rate of 67 g/min, screw speed of 500 rpm and feed moisture of 17% were kept constant during the extrusion experiments. The barrel temperature profile was: 40, 70, 75, 90, 95, 110 and 110 °C in sections 1–6 and in the die. Two water addition regimens were used: in barrel–water feed (IB) and preconditioning (PC). Preconditioning was carried out by adjusting the moisture content of the bran-flour mixtures before extrusion using the protocol published by (Alam et al., 2014). The values of torque, die pressure and die temperature were monitored and recorded during the extrusion.

2.4. Macrostructural analyses

The extruded samples of each extrusion experiment were collected, dried at 105 °C for 20 min and cooled to room temperature. The measurements of expansion rate, specific length and piece density were made from 20 replicates from each extrusion treatments using the method described by Alam et al. (2014).

2.5. Microstructural analyses

In X-ray microtomography (XMT), the samples were analysed in triplicate using a method described by Alam et al. (2014). Each sample of 10 mm long pieces were scanned using a desk-top XMT system (Model 1172, SkyScan, Aartselaar, Belgium) to obtain the parameters of porosity, average cell wall thickness (t), average cell diameter (D) and average cell area. Average cell area was calculated using the formula (area = $(\pi/4) \times D^2$), where D represents average cell diameter.

2.6. Characterization of textural properties

Textural properties of extrudates were analysed by the uniaxial

Table 1
Experimental plan for product optimization.

Sample	Median particle size (μm)	Hydration regimen (IB/PC)	Bran (%)
Coarse IB 15%	440	IB	15
Coarse IB 30%	440	IB	30
Coarse PC 15%	440	PC	15
Coarse PC 30%	440	PC	30
Fine IB 15%	28	IB	15
Fine IB 30%	28	IB	30
Fine PC 15%	28	PC	15
Fine PC 30%	28	PC	30

Raw material concentration in feed based on dry mass (*IB* = in barrel–water feed, *PC* = preconditioning).

compression test using a texture analyser (Texture Analyser TA-HDi, HD3071, Stable Micro Systems, United Kingdom) equipped with a 250 kg load cell and a cylindrical 36 mm aluminium probe using a protocol used by Alam et al. (2014). The samples (20 replicates for each experiment) were deformed at 70% strain with a test speed of 1 mm/s. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain values of hardness (F_{max}), crushing force, crispiness work (C_w) and crispiness index (C_i). The calculation was performed using the formulas published by Alam et al. (2014). High crispiness is accompanied by a high C_i and low C_w value, whereas low crispiness corresponds to a low C_i and high C_w value.

2.7. Starch hydrolysis index analyses

In vitro starch hydrolysis index (HI) was measured using a method described by Sozer et al. (2014). The ground extruded samples of about 2 g was used to obtain each starch hydrolysis curve. The area under the curve (A_{uc}) was obtained after 180 min incubation with porcine pancreatic α-amylase (Sigma-Aldrich Co. LLC, USA). The A_{uc} was calculated using Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA, USA) programme with the preloaded macros. HI values were calculated using the formula: HI = (A_{uc} of extrudates / Average A_{uc} of white wheat bread) × 100 and the results reported as mean ± SD. Whole grain rye bread and extrudates made of 100% coarse and fine rye bran was analysed as control sample.

2.8. Water absorption and water solubility analyses

Water absorption index (WAI) and water solubility index (WSI) were measured using a method described by Anderson et al. (1969) with modifications. WAI measures the amount of water absorbed by starch and WSI measures the amount of soluble components released from starch. Extruded samples were ground by centrifugal mill (Retsch ZM200, Haan, Germany) to pass through 250 μm sieves. Extruded samples of 2.5 g was suspended in 25 ml of deionized water and mixed shortly on a vortex mixer. Sample was hydrated for 30 min at room temperature using a planar-shaker (model B1, Edmund Buhler, Tübingen, Germany) and centrifuged (Heraeus Sepatech Biofuge 28RS, Heraeus, Osterode, Germany) at 3000 × g at room temperature for 10 min. The supernatant was separated and the weight of sediment was obtained. The amount of dissolved solids was determined by drying the supernatant at 130 °C for 3 h and by obtaining the weight of dried sample. Measurements were made in triplicate.

Water absorption index (WAI) was calculated as follows:

$$WAI = \frac{m_\beta}{m_\alpha} \times 100$$

where m_β is the weight of the sediment (in gram, g) and m_α is the weight of the sample (g).

Water solubility index (WSI) was calculated:

$$WSI = \frac{m_\gamma}{m_a} \times 100$$

where m_γ is the weight of the dissolved solids in supernatant (g) and m_a is the weight of the sample (g).

2.9. Statistical analyses

Statistical significance was assessed by one-way ANOVA (Tukey's honest significant difference (HSD)) using a significance level of 0.05. Linear correlations between different variables were calculated using 2-tailed Pearson bivariate correlation with significance levels of 0.01 and 0.05. Statistical analyses were made using SPSS Statistics 20 (SPSS Inc., Chicago, USA) software.

3. Results and discussion

3.1. Macrostructure

Decrease in bran particle size from 440 to 28 μm increased expansion rate but decreased specific length and piece density of rye bran enriched starch based extrudates. Expansion rate ranged between 281% and 323% and density from 187 kg/m^3 to 229 kg/m^3 and negatively correlated with each other ($r = -0.88$, $P < 0.01$) (Fig. 1 and Table 3). This is in agreement with previous literature where particle size reduction of corn bran (Pai et al., 2009), sugar beet fibre (Lue et al., 1991) and rye bran (Alam et al., 2014) resulted in significant increases in expansion. There was significant positive correlation ($r = 0.74$, $P < 0.05$) between the particle size and specific length. Specific length varied from 25.7 to 30.6 m/kg and showed a negative correlation ($r = -0.83$, $P < 0.05$) with expansion rate indicating that the expansion rate increased with decreasing specific length (Fig. 1). A significant negative correlation ($r = -0.81$, $P < 0.05$) between the particle size and expansion rate indicating that the higher the bran particle size the lower the radial expansion, which is in agreement with the study of Santala et al. (2014) of wheat bran extrudates. As part of the starch is replaced by bran particles, the extensibility of the melt in the radial direction is reduced. Bran particles align longitudinally due to the shear in the extruder and hinder radial expansion, which would be against the orientation of the fibres and cause rupture of the cells. Thus, radial expansion decreases and is compensated with longitudinal expansion.

Increase in bran content from 15% to 30% decreased expansion and increased piece density significantly ($p < 0.05$). Addition of bran (e.g. wheat, corn and oat) or other fibre sources (e.g. apple pomace) has often been reported to decrease expansion rate and increase density and specific length (Brennan et al., 2008; Robin et al., 2012; Sibakov et al., 2014). It is believed that above a critical concentration (10–15%), fibres start to interfere with the

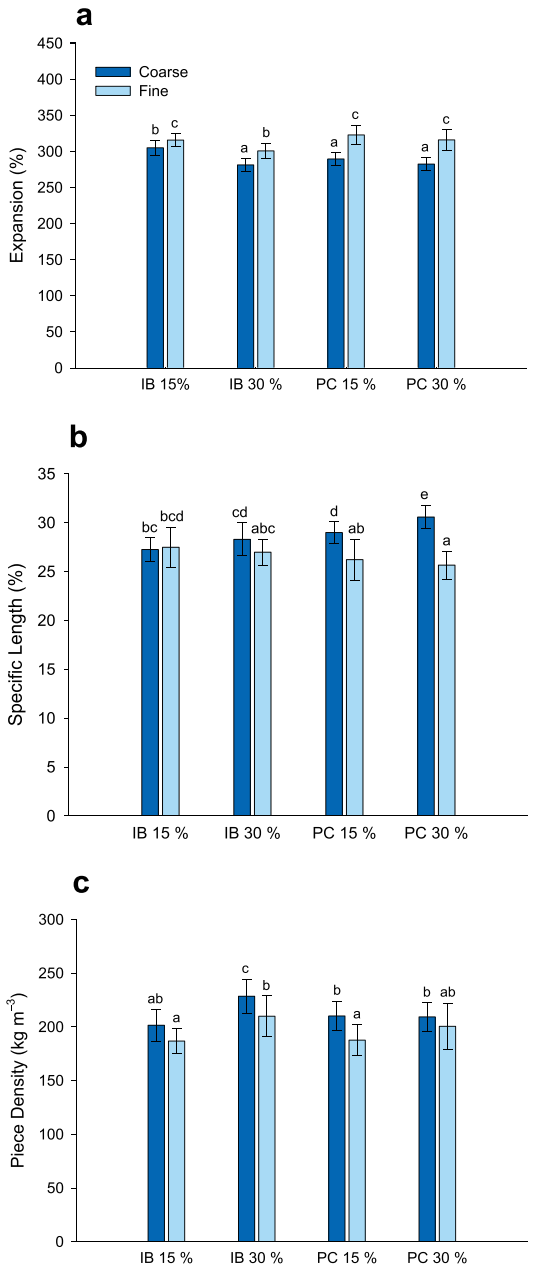


Fig. 1. a) Expansion, **b)** specific length and **c)** piece density of the extrudates made with coarse-, and fine-particle-sized of rye bran (15% and 30% inclusion) processed with 17% feed moisture in two different hydration regimens (*IB* in barrel–water feed, *PC* preconditioning) at 500 rpm and at 110 °C. Bars followed by the **same letters** in **each figure** were **not** significantly different ($P < 0.05$).

continuous structure of the melt and prevent its elastic deformation and reduce gas holding capacity during expansion (Sozer and Poutanen, 2013). A recent study by Chanvrier et al. (2014) reported a significant loss of expansion to occur only when more than 20% wheat and oat bran were added into whole wheat and corn based recipes.

A pronounced effect of pre-conditioning on expansion rate was observed particularly for fine-particle sized bran at 30% addition level. Preconditioning gave more expanded extrudates as compared to in-barrel water fed samples (IB). Liu et al. (2011) mentioned that typically a higher IB water addition resulted in lower expansion. This is related to reduction of melt viscosity by decreasing the temperature which corresponds to more cell collapse. However, effect of hydration regimen on expansion was rather complex for coarse-particle sized bran; a reduced expansion was found for pre-conditioned samples. It could be assumed that coarse-particle sized bran had greater water binding capacity and bound more water during preconditioning and thus reduced the amount of water available for expansion.

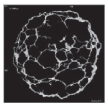
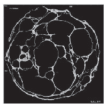
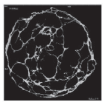
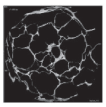
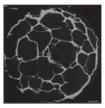
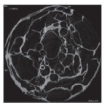
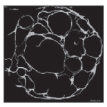
3.2. Microstructure

Microstructural properties were studied by x-ray microtomography (Table 2). Particle size reduction of bran has no significant effect on the porosity and air cell diameter. Due to the high amount of starch (74–82%), porosity of extrudates was high, ranging between 86% and 90%. Average cell wall thickness (t) ranged between 0.14 and 0.19 mm. Particle size reduction influenced t with only IB hydration regimen at 30% bran addition level. Average cell diameter ranged between 1.1 and 1.4 and 1.0–1.6 mm, whereas cell area values ranged between 1.0 and 1.5 and 0.9–2.0 mm^2 for coarse- and fine-particle sized bran extrudates, respectively. Both cell diameter and area varies with the expansion at the die exit. The bubble formation at the die exit occurs due to differences in in-barrel and ambient pressure and is mainly spontaneous which ultimately creates large structural variations in between replicates. Among microstructural parameters only cell wall thickness significantly increased by particle size reduction. Overall, particle size reduction, bran concentration and hydration regimen had no significant impact on the microstructural properties of the extrudate. This observation is in contrast with the results reported by Chanvrier et al. (2014). They showed that increasing amount of wheat bran (10–32%) and oat bran concentrate (10–26%) in whole grain wheat extrudates decreased porosity and average cell size when the total dietary fibre increased from 5 to 17%. In their studies bran was used in mixture with whole wheat, refined wheat and corn flours. Our current study indicates that there is a critical bran concentration below which particle size reduction of the bran does not influence micro-structural properties.

3.3. Textural properties

Structure and texture of snack foods are important for product quality and consumer acceptance. For extruded puffed snacks, expansion rate and crispiness are the most important quality parameters. Textural properties (e.g. hardness, crushing force, crispiness work and crispiness index) of the rye extrudates containing bran are shown in Fig. 2. Bran particle size had no significant effect on hardness (i.e., maximum force) but it showed positive correlation with the crushing force (i.e., average force) and crispiness work of extrudates. These results indicate that extrudates with coarse rye bran were less crispy (C_w: 2.9–3.3 N mm) than extrudates with fine bran (C_w: 1.7–2.6 N mm), except IB extrudates. Crispiness indices were significantly influenced by bran particle size. Addition of fine rye bran increased crispiness compared to the extrudates with

Table 2
Microstructural parameters of extrudates containing coarse and fine particle-sized-rye bran.

Particle size	Sample	Representative image	Porosity (%)	D (mm)	t (mm)	Cell area (mm^2)
Coarse (440 μm)	IB 15		90 ± 1.1 [a]	1.4 ± 0.07 [ab]	0.14 ± 0.01 [a]	1.55 ± 0.15 [ab]
	IB 30		86 ± 1.1 [a]	1.1 ± 0.06 [ab]	0.16 ± 0.01 [ab]	1.02 ± 0.11 [ab]
	PC 15		89 ± 2.7 [a]	1.3 ± 0.22 [ab]	0.14 ± 0.01 [a]	1.36 ± 0.44 [ab]
	PC 30		87 ± 0.6 [a]	1.2 ± 0.07 [ab]	0.14 ± 0.00 [a]	1.12 ± 0.12 [ab]
Fine (28 μm)	IB 15		89 ± 1.5 [a]	1.5 ± 0.18 [ab]	0.17 ± 0.01 [bc]	1.73 ± 0.40 [ab]
	IB 30		89 ± 1.2 [a]	1.6 ± 0.11 [b]	0.19 ± 0.01 [c]	2.06 ± 0.29 [b]
	PC 15		88 ± 2.2 [a]	1.0 ± 0.36 [a]	0.15 ± 0.01 [ab]	0.86 ± 0.63 [a]
	PC 30		88 ± 2.7 [a]	1.3 ± 0.28 [ab]	0.16 ± 0.00 [abc]	1.41 ± 0.54 [ab]

D = Average cell diameter, t = Average cell wall thickness.
IB = in barrel–water feed, *PC* = preconditioning, 15 and 30% refers to the bran concentration.
Values followed by the **same letters** in the **same column** were **not** significantly different ($P < 0.05$).

coarse rye bran (Fig. 2). This suggests that a decrease in bran particle size allows higher bran addition without significant changes in product crispiness. Particle size was positively correlated with crushing force ($r = 0.83$, $P < 0.05$), crispiness work ($r = 0.85$, $P < 0.01$) and negatively with crispiness index ($r = -0.80$, $P < 0.05$). These correlations clearly indicate that the effect of particle size on the product texture was significant, and textural properties can be improved by reducing particle size of rye bran. However, extrudates processed with 30% fine-particle sized bran at IB hydration regimen were significantly crispier ($P < 0.05$) than the other extrusion recipes. This indicates that the combination of particle size reduction and selected hydration regimen i.e., in-barrel water feed could improve the textural properties of the fibre snacks. Our finding is in agreement with the result reported by Karkle et al. (2012), who showed that 28% fibre (apple pomace) addition at 17.2% IB improved textural properties of corn-based extrudates by increasing crispiness.

Apparently, the effect of bran concentration on extrudate textural parameters is highly dependent on raw materials and processing conditions. In the current study, regardless of hydration regimen, increase in bran concentration from 15 to 30% increased hardness and decreased crispiness of the rye extrudates. Similar results were also reported by other researchers; e.g. 5–20% inclusion of wheat or oat bran significantly increased hardness and decreased crispiness of oat and wheat flour extrudates (Sibakov et al., 2014; Brennan et al., 2008). Robin et al. (2011a) found that extrusion of a mixture of wheat bran and wheat flour (to achieve fibre content of 24.4%) produced harder extrudates. Harder and less crispy extrudates were due to finer cellular structure and higher cell density of the bran-containing extrudates.

3.4. Starch hydrolysis index

Increasing total dietary fibre (TDF) content from 8.2% to 12.6%

Table 3
Pearson's correlation matrix for macro-, microstructural and mechanical properties.

Variables	Correlations															
	Particle size	Bran conc.	Hydration regimen	Expansion rate	Specific length	Piece density	WAI	WSI	Porosity	Avg. Cell diameter	Avg. Cell wall thickness	Avg. Cell area	Hardness	Crushing force	Crispiness work	Crispiness index
Particle size (μm)	1	0.000	0.000	**−0.808***	**0.743***	0.638	−0.375	0.272	−0.114	−0.257	−0.706	−0.360	0.057	**0.825***	**0.845****	**−0.800***
Bran concentration (%)		1	0.000	−0.440	0.131	0.613	0.545	−0.649	−0.644	0.054	0.464	0.054	0.625	−0.185	−0.400	0.171
Hydration regimen (IB/PC)			1	0.067	0.121	−0.189	**0.708***	−0.640	−0.207	−0.553	−0.443	−0.529	−0.693	0.189	0.058	−0.187
Expansion rate (%)				1	**−0.833***	**−0.876****	0.078	0.120	0.333	0.082	0.248	0.171	−0.475	−0.452	−0.438	0.439
Specific length (mkg^{-1})					1	0.466	−0.030	−0.085	−0.152	−0.140	−0.432	−0.234	0.190	0.561	0.592	−0.515
Piece density (kgm^{-3})						1	−0.063	−0.150	−0.462	−0.063	−0.026	−0.122	0.585	0.248	0.184	−0.270
WAI							1	**−0.964****	−0.493	−0.228	0.212	−0.183	−0.184	−0.247	−0.455	0.207
WSI								1	0.499	0.147	−0.275	0.113	0.011	0.293	0.480	−0.252
Porosity (%)									1	0.628	0.018	0.629	−0.142	−0.258	−0.012	0.307
Avg. cell diameter (mm)										1	0.622	**0.993****	0.497	−0.564	−0.406	0.553
Avg. cell wall thickness (mm)											1	0.680	0.638	**−0.891****	**−0.894****	**0.866****
Cell area (mm^2)												1	0.475	−0.647	−0.498	0.636
Hardness (N)													1	−0.330	−0.324	0.356
Crushing force (N)														1	**0.952****	**−0.984****
Crispiness work (N mm)															1	**−0.934****
Crispiness index ($\times 10^{-4}$)																1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

significantly ($P < 0.05$) reduced *in vitro* starch hydrolysis of the extrudates containing 30% fine bran. HI of the extrudates containing coarse bran (Table 4), however, was not significantly different within different bran concentration. Although the gradual decrease in the amount of starch was similar for coarse (80.9 → 74.1%) and fine bran (81.9 → 75.8%) extrudates due to the increased bran level, the decrease in HI was observed only for 30% fine bran extrudates. Also extrudates made from 100% fine rye bran with higher starch amount had significantly lower HI compared to 100% coarse rye bran extrudates (Table 4), which correspond to the results found with 30% fine bran extrudates. Identical HI value was obtained for both 30% and 100% fine bran extrudates even though the starch amount was 30.2% higher in 30% bran added extrudates. Therefore, it could be hypothesised that HI of fine bran extrudates was not only affected by the starch amount but also by microstructural architecture. However, all extruded samples had lower HI than wholegrain rye bread regardless of starch amount.

High shear forces in the extruder barrel disrupt the structural integrity of starch granules and make them susceptible for enzymatic attack (Anguita et al., 2006; Altan et al., 2009). In earlier work, wheat bran, inulin and guar gum supplementation in extruded products at TDF levels of 5–15% reduced HI (Brennan et al., 2012); similar trend of reduced HI was obtained for grape and tomato pomace (Altan et al., 2009) as well as apple pomace (Karkle, 2011) supplemented extrudates with TDF levels of 15–21% and 1–23%, respectively. Adding soluble DF (such as inulin) and insoluble DF (such as wheat bran) gave slightly lower *in vitro* starch digestibility rate (Brennan et al., 2008).

In this study, 30% fine rye bran (TDF: 12.6%) extrudates had significantly ($P < 0.05$) thicker cell walls compared to 15% fine rye bran extrudates (Table 2). In our previous study, significantly ($P < 0.05$) higher cell wall thickness was observed for 100% fine bran extrudates (0.32 ± 0.04 mm) than 100% coarse bran extrudates (0.24 ± 0.01 mm) (Alam et al., 2014). Increased cell wall thickness might have reduced starch digestibility for 30% fine rye bran extrudates as large amount of starch granules could not come into contact with enzyme and remained intact. Anguita et al. (2006) found that particle size reduction (3000 → 800 μm) slightly increased starch hydrolysis rate of corn and wheat extrudates with crude fibre content of 2.4 and 3.3%, respectively. However, in the current study significant ($P < 0.05$) increase in the HI due to particle size reduction observed for only 15% bran addition level. Inverse relationship between starch digestibility and fibre addition level was pronounced only in case of fine rye bran extrudates. Brennan et al. (2008) showed that addition of either guar gum or wheat bran giving a TDF content of up to 15% still had high starch hydrolysis rate, which is in accordance with the results presented here.

3.5. Water absorption and solubility

Particle size reduction and increase in bran concentration increased water absorption (WAI) of extrudates, but the effect on water solubility (WSI) was opposite (Fig. 3). Fine rye bran extrudates resulted in higher WAI compared to coarse counterparts. Coarse rye bran contained larger pieces of aleurone layer than finely milled bran, which might reduce the rate of water penetration. Reduction of wheat bran particle size was also reported to increase WAI with increased ratio of soluble dietary fibre (SDF) to IDF (Zhu et al., 2010). In the current study, fine rye bran contained slightly higher amount of starch (44.8 vs. 38.4%) and SDF (5.5 vs. 4.9%) than coarse bran, which might have promoted higher WAI values. Comparatively lower amount of starch in coarse bran reduced the water swelling capacity and may thus have resulted in lower water absorption. Singh et al. (2007) pointed out that higher

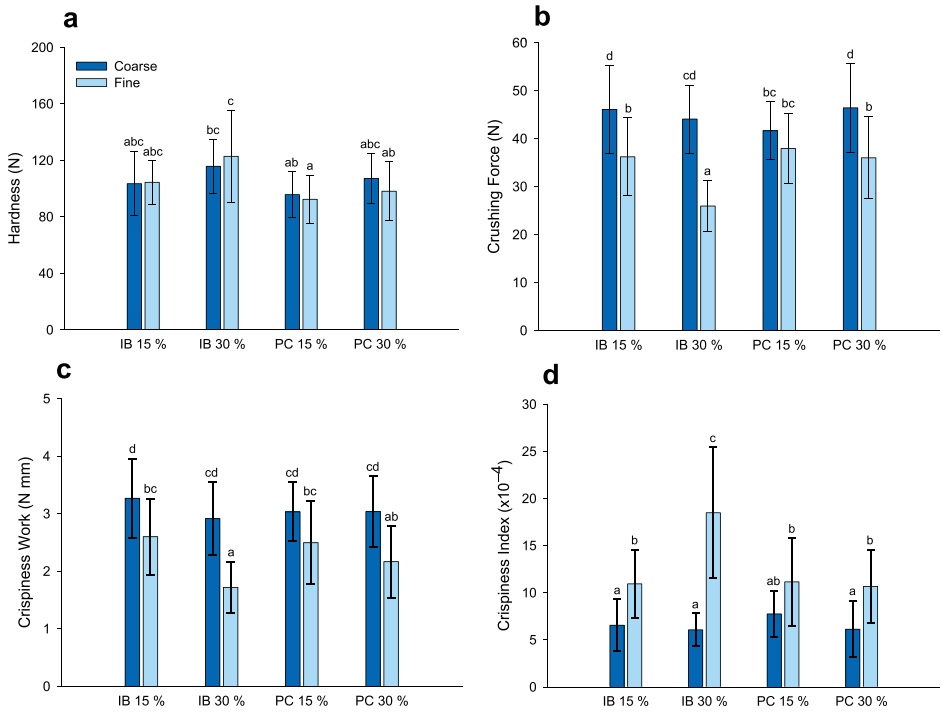


Fig. 2. **a)** Hardness, **b)** crushing force, **c)** crispiness work and **d)** crispiness index of the extrudates made with coarse-, and fine-particle-sized rye bran (15% and 30% inclusion) processed with 17% feed moisture in two different hydration regimens (*IB* in barrel–water feed, *PC* preconditioning) at 500 rpm and at 110 °C. Bars followed by the **same letters** in **each figure** were **not** significantly different ($P < 0.05$).

Table 4
Hydrolysis index (values are mean of three replicates) and starch content of extrudates containing coarse and fine particle-sized-rye bran.

Sample	HI	Starch (%, dry weight basis)
Coarse 15%	70.3 ± 1..9 [a]	80.9 ± 0.4 [g]
Coarse 30%	66.9 ± 1.7 [a]	74.1 ± 0.3 [e]
Coarse 100%	75.4 ± 0.8 [b]	40.2 ± 0.1 [a]
Fine 15%	74.6 ± 1.5 [b]	81.9 ± 0.9 [g]
Fine 30%	69.0 ± 1.9 [a]	75.8 ± 0.4 [f]
Fine 100%	68.9 ± 1.9 [a]	45.6 ± 0.2 [b]
Wholegrain rye bread	80.8 ± 0.9 [c]	57.1 ± 0.6 [c]
White wheat bread	100.0 ± 0.0 [d]	70.0 ± 0.4 [d]

All extruded samples were processed with *IB* in barrel-water feed.
Values followed by the **same letters** in the **same column** were **not** significantly different ($P < 0.05$).

amount of starch in the feed material absorbs more water when gelatinized during extrusion, even at lower water (12–22%) level.

Increased bran addition increased WAI and decreased WSI. Similar results were also reported in other studies, where rice bran and wheat bran were added into the feed material (Robin et al., 2011b; Charunuch et al., 2014). Increases in bran concentration increased WAI due to the presence of higher amount of hydrophilic fibre particle. Higher level of bran addition might cause some structural modification in bran at high screw speed. High screw speed produces high specific mechanical energy inside the barrel and could probably induce a more open structure of fibre which allows more water to come in contact with hydrophilic groups. A negative correlation ($r = 0.96$, $P < 0.01$) was observed between WAI and WSI regardless of either bran addition level or particle size reduction. A negative correlation between WAI and WSI was also shown, regardless of wheat bran concentration (2.8%, 12.6% and 24.4%), in extrusion of wheat flour-bran mixture (Robin et al., 2011b). In preconditioned samples bran had more time to hydrate before extrusion leading to more complete gelatinization of starch and swelling of the fibres and thus to significant ($P < 0.05$) increase of WAI.

4. Conclusions

This study focused on starchy extrudates with added rye bran of two different particle sizes (coarse: 440 μm and fine: 28 μm) and showed that up to 30% of fine rye bran could be added without interfering with structural and textural properties. In barrel-water feeding improved the textural properties and reduced the *in vitro* hydrolysis index of 30% fine bran added extrudates, while preconditioning improved only the expansion rate. Increase in WAI, expansion and crispiness with decreased piece density and WSI was observed with particle size reduction, which however had no significant effect on hardness. Particle size reduction of bran had very minor effects on the porosity and cell diameter of the extrudates but cell wall thickness and cell area increased with reduced particle size. Hydrolysis index was slightly smaller at 30% addition of fine bran, but all the other extrudates had quite readily accessible starch. Extrudates supplemented with fine bran had better structural and textural properties than with coarse bran but there was minor influence on *in vitro* HI.

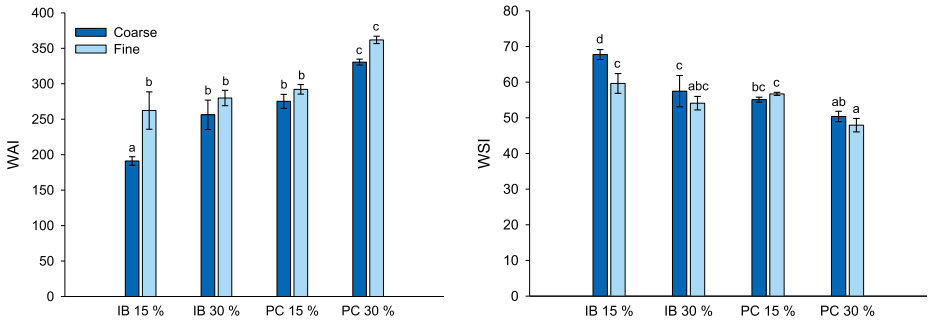


Fig. 3. Water absorption index (WAI) and water solubility index (WSI) of extrudates made with coarse-, and fine-particle-sized of rye bran (15% and 30% inclusion) processed with 17% feed moisture in two different hydration regimens (*IB* in barrel–water feed, *PC* preconditioning) at 500 rpm and at 110 °C. Bars followed by the **same letters** in **each figure** were **not** significantly different ($P < 0.05$).

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PUBLICATION III

Effects of structural and textural properties of brittle cereal foams on mechanisms of oral breakdown and *in vitro* starch digestibility

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Effects of structural and textural properties of brittle cereal foams on mechanisms of oral breakdown and *in vitro* starch digestibility

Syed Ariful Alam *, Saara Pentikäinen, Johanna Närväinen, Ulla Holopainen-Mantila, Kaisa Poutanen, Nesli Sozer *

VTT Technical Research Centre of Finland Ltd., Tietotie 2, P.O. Box 1000, FI-02044, VTT, Finland

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ABSTRACT

Structural and textural properties as well as the dietary fibre content of solid cereal foams influence the oral breakdown of structure, bolus formation and digestibility. The aim of this study was to investigate how structural differences of solid cereal foams (puffs vs. flakes) affect *in vivo* chewing and *in vitro* starch digestion. Four extruded puffs and flakes were produced from endosperm rye flour by extrusion processing without or with 10% rye bran (RB) addition. Extruded puffs and flakes were masticated by fifteen healthy females and the process was monitored using electromyography. Extruded puffs were more porous than flakes (97% vs 35%). The two products were also significantly different ($p < 0.05$) in their structural and textural properties such as expansion, hardness, density and crispiness. A negative correlation was observed between hardness and crispiness index ($p < 0.05$, $r = -0.950$) and density and porosity ($p < 0.05$, $r = -0.964$). Addition of 10% RB had a significant effect on structural, textural and mastication properties both for puffs and flakes. Mastication of puffs required less total work than flakes (204 vs. 456%) and they were degraded to smaller particles than flakes during mastication. Irrespectively of the considerable differences in structure, texture and oral disintegration process, no significant ($p < 0.05$) differences were observed between puffs and flakes (86.4 vs. 85.1) in terms of starch hydrolysis index. RB addition increased the hydrolysis index of puffs and flakes to 89.7 and 94.5, respectively, which was probably attributable to the increased number of particles in the bolus.

1. Introduction

Consumption of snack food products is globally high; the market for extruded products alone is expected to reach $31 billion by 2019 (Markets and Markets, 2014). Consumer interest in extruded products is growing due to their on-trend texture and shape. However, extruded cereal snacks are often unhealthy due to their excessive starch content and lack of dietary fibre (Alam et al., 2016). Extruded products have the structure of solid foam. Open brittle foams such as extruded puffs consist of an interconnected porous structure with thin cell walls, offering a mechanically weaker and less dense structure compared to closed and dense structures such as extruded flakes (Dogan, Romero, Zheng, Cuitino, & Kokini, 2008).

Food macrostructure (such as porosity and relative density) and microstructure (cell diameter, cell distribution and cell wall thickness) play an important role in mastication, bolus formation, transportation of the bolus in the human gastrointestinal tract and in starch hydrolysis (Hoebler et al., 1998; Kong & Singh, 2008; Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre, 2013). Generally, the addition of insoluble fibre (e.g. bran) to extruded matrices interferes with the macro-, micro- and textural properties by decreasing the expansion and crispiness and increasing hardness, cell wall thickness and density (Alam et al., 2014; Guan, Fang, & Hanna, 2004; Lue, Hsieh, Peng, & Huff, 1990; Mendonça, Grossmann, & Verhé, 2000; Moore, Sanei, Hecke, & Bouvier, 1990). These effects are more pronounced for coarse particle sized bran, particularly at higher addition levels (Alam et al., 2016).

Food is mechanically disintegrated to smaller fragments and enzymatically broken down by digestive enzymes during digestion (Bornhorst & Singh, 2012; Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Panouillé, Saint-Eve, Déléris, Le Bleis, & Souchon, 2014; Stokes, Boehm, & Baier, 2013). Oral processing of food, i.e. mastication, prepares the food into a lubricated and cohesive bolus by means of complex mechanical and chemical transformation (Le Bleis et al., 2013).

Textural properties of cereal products, such as hardness and crispiness, are also assumed to have a role in physiological responses (Kong & Singh, 2008; Turgeon & Rioux, 2011). Agrawal, Lucas, Prinz, and Bruce (1997) reported that the fracture events occurring during mastication of food (raw vegetables, nuts and cheese) are inversely related to the products' hardness; however the exact food properties determining mastication are not fully understood. The bolus is further broken down

* Corresponding authors.
E-mail addresses: ariful.alam@vtt.fi (S.A. Alam), nesli.sozer@vtt.fi (N. Sozer).

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in the stomach by gastric secretions and muscular contractions and gradually released to the duodenum, when the particle size is <1–2 mm (Thomas, 2006). The particle size distribution of a bolus depends on the original food structure and texture (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000; Jalabert-Malbos et al., 2007). Crispy and less hard products disintegrate more easily during mastication, thus producing smaller particles, whereas hard and less crispy products result in larger particles in the bolus (Pangborn & Lundgren, 1977). The textural properties of solid foams are related to matrix architecture, its composition, and the state and homogeneity of the solid matrix (Sozer, Bruins, Dietzel, & Kokini, 2011). However, the literature on how structural and mechanical properties of different brittle solid foams affect mastication, bolus formation and digestibility is scarce and is focused on commercial flakes made from refined flour (Hedjazi, Guessasma, Yven, Della Valle, & Salles, 2013; Hedjazi, Martin, Guessasma, Della Valle, & Dendievel, 2014; Yven, Guessasma, Chaunier, Della Valle, & Salles, 2010).

We have previously studied the *in vitro* starch digestibility and the structural and textural properties of rye extrudates (Alam et al., 2016), and showed that microstructure has a major role in the digestibility of high fibre extruded puffs. The aim of this study was to elucidate how structural differences in open and closed extruded rye matrices influence oral processing and *in vitro* starch digestibility. We hypothesised that closed solid cereal foams (flakes) prepared by extrusion would require more mastication, disintegrate into larger particles and have a lower hydrolysis index compared to open cereal foams (puffs).

2. Material and methods

2.1. Puffs and flakes

2.1.1. Feed material preparation

Rye endosperm flour (ERF) obtained from Helsingin Mylly Oy (Järvenpää, Finland) was used as base material during the extrusion processing. Commercial rye bran (RB) was purchased from Fazer Mills and Mixes Ltd. (Lahti, Finland) and milled to obtain a particle size of $D_{50} < 30$ μm. RB was ground by ultra-fine grinding equipment Ceren Miller DAU (Masuko Sangyo Ltd. Co., Kawaguchi, Japan). Directly puffed extruded snacks and flakes were prepared either using 100% ERF or with 10% RB added to increase the fibre content. Salt (0.8%) was added in the dry mix of all recipes. A spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany) was used to mix the raw material for 8 min. For each extrusion trial, a 2 kg mixes from each recipe was used.

2.1.2. Particle size analysis

Particle size of the ultra-fine milled RB was analysed by a Beckman Coulter LS 230 (Beckman Coulter Inc., CA, USA) using a liquid module and ethanol as a carrier. The D_{50} and D_{90} values of the RB materials were: 23.7 ± 0.8 and 155.5 ± 9.2 μm, respectively.

2.1.3. Extrusion processing

Extruded puffs and flakes were prepared using a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd., Peterborough, UK) with a constant feed rate of 60 g/min and a temperature profile increasing gradually from 80 to 120 °C from feed inlet to die exit in the following order: 80–95–110–120 °C. For feeding of the flour mix a co-rotating twin screw feeder, K-Tron Soder, Niederlenz, Switzerland was used. Twin screw configuration with a 3 mm die was used in all extrusion trials. Feed rate calibration was performed for each recipe before running the extruder. Water was pumped into the extruder barrel in order to obtain desired moisture contents in the extrudates. Extruded products were collected continuously from the exit die (diameter 3 mm) and dried immediately in an oven, 30 min for puffs and 60 min for flakes at 100 °C. Recipe and extrusion process parameters are shown in Table 1. The extrusion trial was repeated for extruded puffs and flakes to observe whether extrusion processing affects the product quality if extruded in a different batch. The torque values were monitored and recorded during the extrusion. Specific mechanical energy (SME) was calculated using Eq. (1):

$$SME\left(\text{kW h kg}^{-1}\right) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (1)$$

where ω is the screw speed (rpm), ω_r is the maximum screw speed of the extruder used (500 rpm), τ is the torque (%), Z_r is the maximum power capacity of the extruder (2 kW) and Q is the feed rate (kg/h).

2.2. Starch content and moisture

Analyses of the starch content of the extruded samples were made using total starch by the AACC method no. 76.13 (AACC, 1999), insoluble and soluble dietary fibre by the AOAC method no. 991.43 (AOAC, 1992), and moisture content by drying the samples in an oven at 130 °C for 2 h. Total starch and moisture content analysis were performed as triplicates.

2.3. Structure and texture analysis

2.3.1. Macro- and microstructure

The extruded samples of each extrusion experiment were collected, dried at 100 °C for 30 min for puffs and 45 min for flakes, and cooled to room temperature. The measurements of expansion rate, specific length and piece density were made from 20 replicates from each extrusion treatment using the method described by Alam et al. (2014). Microstructures of the extruded puffs and flakes were analysed using a desktop X-ray micro tomography (XMT) system (Model 1172, SkyScan, Aartselaar, Belgium) and a method described by Alam et al. (2014). Puffed extruded samples were made by cutting 10 mm pieces from the extrudate samples with an electric saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan) and the flaked samples were analysed as is. The diameter of the puffs was approximately 16–17 mm. A desktop XMT system (Model 1172, SkyScan, Aartselaar, Belgium) with a 12-bit cooled CCD camera (512 × 1024 pixels) was used to collect the X-ray data. The x-ray tube was operated at a voltage of 40 kV and a current of 250 μA in order to obtain optimum contrast between air cells and cell walls. The samples were rotated a total of 180° during the scanning process with a voxel size of 11.65 μm × 11.65 μm × 11.65 μm. The total scanning time was 18 min. The initial X-ray radiographs or raw images were obtained at every 0.7° of rotation. After scanning, reconstructed images were further analysed for total, open and closed porosity (%), cell wall thickness (mm) and cell diameter (mm) by Ctan (v. 1.12, Skyscan, Belgium) image analysis software. Five different measurements from different XMT samples were performed for both puffs and flakes and the results were reported as means ± SD. Stereomicroscopic images were captured using the protocol published by Nikinmaa et al. (2016).

2.3.2. Texture

Texture analysis of extruded puffs was performed by uniaxial compression of the extrudates by using a TA.XT2 Texture analyser (Stable Micro System Ltd., Godalming, United Kingdom) equipped with a 30 kg load cell and 25 mm diameter cylinder aluminium probe. Puffed samples were prepared by cutting the extruded samples to 10 mm height. The samples were deformed at 70% strain with a test speed of 1 mm/s and an acquisition rate 200 points/s. The force-deformation curve was obtained to assess the textural properties of the snack samples. The analysis was performed with 30 replicates. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain values of maximum force, actual curve length, area under the force-deformation curve and number of peaks. The number of peaks represents the number of cell wall ruptures during compression and hardness is the maximum force needed to initiate cell wall crack. Hardness (F_{max}), crispiness work (C_w) and crispiness index (C_i) were calculated using

Table 1
Extrusion recipes and process parameters.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Screw speed (rpm)	345	345	250	260
Torque (%)	86–90	86–92	42	43
Feed rate (g/min)	60	60	60	60
SME ($kW \cdot h \cdot kg^{-1}$)	0.330–0.345	0.330–0.353	0.117	0.124

ERF: endosperm rye flour; RB: rye bran; salt (0.8%) was added to all extrusion recipes.

the protocol published by Alam et al. (2014). High crispiness is accompanied by a high C_i and low C_w value, whereas low crispiness corresponds to a low C_i and high C_w value.

Due to the thin and flat shape of the flakes, the texture analysis protocol used for puffs was not suitable. Instead, the mechanical stress was applied onto a thick bed of flakes using a Texture Analyser TA-HDi (HD3071, Stable Micro Systems, United Kingdom) equipped with a 250 kg load cell and a 5-bladed Kramer shear cell ($90 \times 66 \times 82$ mm^3). Test mode was set to compression and cross head speed was 1 mm/s. The samples were deformed at 25% strain and the acquisition rate was 400 points/s. The weighed amount of the samples (as is) was 35 g (about a 20 mm thick bed in the cell) and 10 replicates were made for each flake sample. The test distance used was 25 mm (cross head return distance was calibrated for 22 mm) in order to ensure that the blades passed through the respective slots of the Kramer shear cell with a clearance of 3 mm. Data obtained from the force-deformation curve were analysed in the same way as described above.

2.4. Mastication trial

2.4.1. Participants

Fifteen young female participants aged between 20 and 40 years were recruited by circulating group emails and through the bulletin boards at the University of Eastern Finland. Only female participants were included in order to avoid unnecessary variation due to the gender effect, as it has been demonstrated in the literature that there is a gender-dependent difference in mastication time (Buschang, Hayasaki, & Throckmorton, 2000). The mean age of participants was 24.6 ± 4.4 years, and their mean Body Mass Index (BMI) was 22.0 ± 1.4 kg/m^2. All participants were of normal weight, they had no missing teeth (except 3rd molars), and they had no diagnosed mastication problems. Smokers were excluded from the study. The participants gave written informed consent to their participation in the study. Ethical principles of good research and clinical practice described in the declaration of Helsinki were followed during the study. Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Northern Savo, Finland.

2.4.2. Procedure

The participants attended one study visit during which all extruded samples were masticated in three replicates. The experiments took place between 8 and 11 a.m., and the participants were instructed to eat breakfast 1 to 1.5 h beforehand. They were familiarised with the study procedure before the actual mastication trial. Four extruded samples of puffs and flakes were offered to each participant in random order. The samples were blind coded by using 3-digit numbers. Each product was served and masticated in three separate portions. Portion sizes represented mouthfuls of food: puff portions consisted of 2 × 3.5 cm (diameter: 1.6–1.7 cm) ribbons and flake portions contained 10.5 g of flakes, presented as 1 tablespoon (thickness: 0.26–0.32 cm and length: 2.2–2.3 cm). The three portions of puffs weighed on average 5.8 ± 0.7 g and puffs with RB weighed 5.3 ± 0.3 g. The participant masticated each portion without swallowing until she felt ready to swallow the portion. Instead of swallowing, the bolus was expectorated to a plastic container which was kept on ice. The three portions each of puffs and flakes samples were masticated in direct succession and between different products there was a break of 2 min during which the mouth was rinsed with water.

2.4.3. Electromyography measurements

The mastication process was characterised by measuring the electrical activity of facial muscles using electromyography (EMG). EMG was measured with the NeurOne system (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. The skin was cleaned with 70% ethanol and bipolar electrodes were placed on the masseter and temporalis muscles on both sides of the face. The muscles were identified by touch when the participant gritted her teeth. EMG activity was measured continuously throughout the whole mastication trial and the data blocks for each chewing period were isolated for analysis by visual inspection and double-checked against the temporal record of the experiment. From the EMG time series, the onset, duration and amplitude of each chewing event was extracted by applying wavelet filtering for the elimination of high frequencies and background fluctuations, followed by squaring and smoothing of the data. Individual chews were recognized from the derivative curve. The parameters extracted for these data were number of chews, chewing time, EMG activity time, duty cycle, force/chew and total work (EMG activity time × force/chew). All EMG data analysis was carried out using Matlab software (The MathWorks Inc., Natick, MA, USA).

2.5. Bolus analyses

2.5.1. Saliva impregnation

Bolus saliva impregnation was determined based on the moisture contents of extruded puffs, flakes and their bolus samples. Wet bolus samples were weighed and placed in an oven at 105 °C overnight and the dried bolus was weighed again. The saliva impregnation was determined by the difference between the water content of boluses and the water content of extruded puffs and flakes.

2.5.2. Particle size distribution

The bolus samples (from eight individuals out of fifteen participants) were diluted into 100 ml of water, mixed with magnetic stirring (220 rpm) for 25 min and left to stand for 5 min in order to allow larger particles to settle to the bottom. Then the turbid liquid containing the smallest particles that could not be imaged was removed and the sample volume was increased with water up to 100 ml. The liquids containing the larger particles were poured onto petri dishes for imaging. Around 6 to 8 petri dishes were needed depending on the sample. The particles were adjusted on petri dishes so that they were as little as possible in contact with each other. Digital images were taken of each petri dish. Particle areas were determined using Cell^P imaging software (Olympus, Germany). The analysis of bolus particle size distribution

(in total: 15 × 4 × 3 = 180 bolus samples) were time consuming thus grouped in three different chew types: six average chewers, one heavy and one light chewer (in total: 8 × 4 × 3 = 96 bolus samples).

2.6. Starch hydrolysis index

In vitro starch hydrolysis index (HI) was determined using a method described by Sozer, Cicerelli, Heiniö, and Poutanen (2014). A ground, extruded puff or flake sample of about 1.5 g (to get 1 g starch in the sample) was used to obtain each starch hydrolysis curve. Two different buffer addition methods (either after 15 min of soaking or directly after adding water) were tested for HI analysis. The latter method was chosen to avoid clump formation. The area under the curve (A_{uc}) was obtained after 180 min incubation with porcine pancreatic α-amylase (A6255, DFP Treated, Type I-A, saline suspension, ≥1000 units/mg protein, Sigma-Aldrich Co. LLC, USA). The A_{uc} was calculated using the Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA, USA) program with the preloaded macro. HI values were calculated by comparing the areas under the curve to that of wheat bread using the formula: HI = (A_{uc} of extrudates/Average A_{uc} of white wheat bread) × 100 and the results were reported as means ± SD.

2.7. Statistical analyses

Overall differences between study products were assessed using one-way ANOVA, and LSD was used as a post hoc analysis for pairwise comparison. Multivariate analysis of variance (MANOVA) was performed in order to distinguish the effects of structural, textural, mastication and bolus properties on the samples, and a separate analysis of variance (ANOVA) was performed with Bonferroni adjustment (or correction) for *p* value. Principal component analysis (PCA) was performed using Oblimin rotation with Kaiser normalization in order to demonstrate the correlation between structural, textural, mastication and bolus properties individually and interaction between all the studied properties. Sampling adequacy was considered acceptable when Kaiser-Meyer-Olkin (KMO) values were over 0.60 and sphericity (Bartlett's *p*) test values were <0.05. Statistical analyses were conducted using SPSS Statistics 20 (SPSS Inc., Chicago, USA) software.

3. Results and discussion

3.1. Structural characteristics

There were notable structural differences between puffs and flakes (Fig. 1, Table 3). Flakes were significantly less porous, less expanded and had higher cell wall thickness compared to puffs (Table 3). Piece density is an indicator of a products' porosity and expansion. Both flakes had higher piece density compared to puffs, and RB addition increased the piece density for both puffs and flakes. Cell diameter was considerably greater (2.4 vs 0.2 mm) for puffs regardless of RB addition (Table 3). Puffs and flakes with added RB had higher fibre content compared to those without RB (Table 2). RB addition in each group (puffs and flakes) did not cause any significant change in microstructural properties such as porosity, average cell diameter and cell wall thickness, but macrostructural properties such as expansion, specific length and piece density were significantly different.

MANOVA results for structural (macro- and microstructure) properties showed that puffs and flakes samples were significantly different ($p < 0.05$) due to the combined effect (Table 4a) of expansion, specific length, piece density, porosity, cell diameter and cell wall thickness. There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.842 and 0.996. Expansion, porosity and piece density including DF had a slightly stronger effect on the extruded samples than specific length, cell diameter and the cell wall thickness. Contrast (simple, K matrix) results showed that all the variables played significant ($p < 0.05$) roles in making the puffs and flakes different, although expansion, porosity and cell diameter did not have significant ($p > 0.05$) effects when comparing 100% ERF flakes vs. 10% RB flakes. Moreover, all macro- and microstructural properties were jointly and individually influenced ($p < 0.05$) by fibre content (Table 4g). The PCA plot shows that a strong positive correlation was found between cell diameter and expansion and between cell

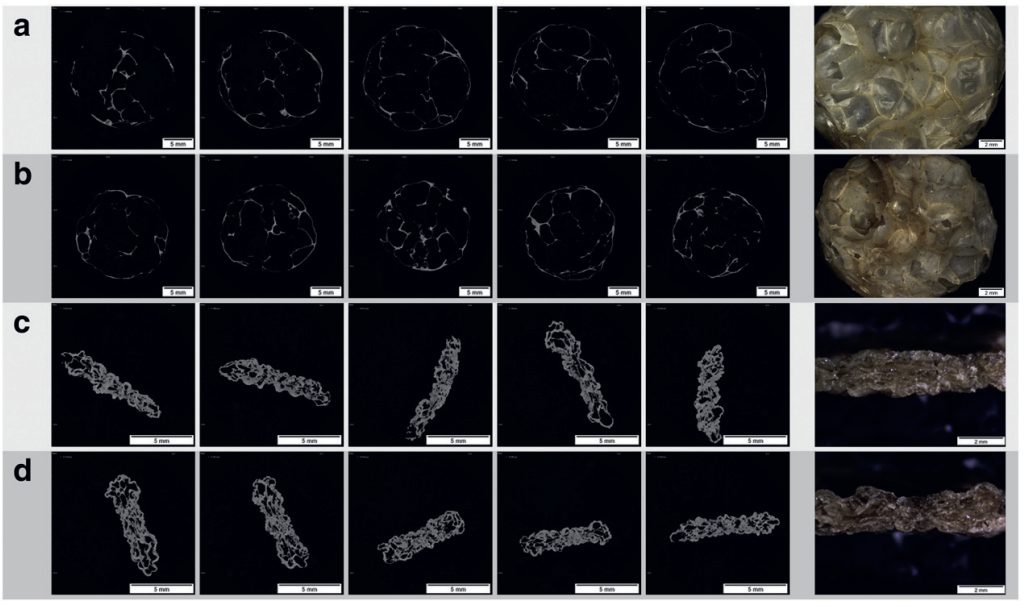

Fig. 1. 2D-XMT (images on the same line are taken from 5 different measurements from different samples; the white bar is 5 mm) and microscopy (the white bar is 2 mm) images of **a)** 100% endosperm rye flour puffs **b)** 90% ERF + 10% rye bran puffs **c)** 100% endosperm rye flour flakes and **d)** 90% ERF + 10% rye bran flakes.

Table 2
Total dietary fibre (TDF), insoluble (IDF) and soluble (SDF) dietary fibre and starch contents of extruded puffs and flakes.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
TDF (%)	5.34	8.00	6.45	8.90
IDF (%)	2.75	5.16	3.95	5.67
SDF (%)	2.59	2.85	2.50	3.23
Starch (%, dry weight basis)	78.17	75.23	76.67	73.22

wall thickness and density (Fig. 2a). Total porosity was positively correlated with expansion and cell diameter, and negatively correlated with piece density and cell wall thickness. On the other hand, increased fibre content increased specific length and piece density and decreased expansion and cell diameter.

3.2. Textural characteristics

Puffs both with or without RB were less hard and crispier (Table 3) than the flakes with similar composition. RB addition increased hardness and reduced crispiness for both puffs and flakes. Puffs and flakes were significantly different ($p < 0.05$) due to the combined (Table 4b) effect of hardness, crispiness work, crispiness index and fibre content. There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.977 and 0.996. The differences between extruded products were more significant in terms of hardness and DF content than crispiness. Contrast results (simple K Matrix) showed that all variables played significant roles ($p < 0.05$) in making the puffs and flakes different, although crispiness index did not have a significant effect when comparing 100% ERF flakes with 10% RB-enriched flakes. Moreover, all textural properties were jointly (Table 4h) and individually influenced by fibre content ($p < 0.05$). The PCA plot shows that hard extruded puffs and flakes were less crispy (Fig. 2b). Increased fibre content increased the hardness and crispiness work and reduced the crispiness index.

3.3. Mastication properties

Flakes required more oral processing compared to puffs, reflected as significantly higher ($p < 0.05$) values in the number of chews, chewing time, EMG activity time, applied force/chew, and total work (Table 5). Samples were significantly different ($p < 0.05$) based on their mastication properties (data obtained from 15 different participants). A higher Wilk's Λ and low η^2 value indicate that although the difference between products observed based on the mastication properties was significant, the effect was not so strong enough to differentiate the products (Table 4c). There was a significant difference ($p < 0.05$) between samples for chewing time, number of bites, EMG activity time and total work, but not for duty cycle and force/chew. Contrast results (K Matrix) showed that all variables except the duty cycle played significant roles ($p < 0.05$) in differentiating the puffs and flakes. However, when comparing 100% ERF flakes and 10% RB-enriched flakes, none of the mastication properties have a strong effect indicating the difference ($p > 0.05$) between them. Moreover, all mastication properties were jointly and individually affected by the fibre content ($p < 0.05$) but force/chews did not show any significant effect (Table 4i). The PCA plot shows that number of chews and chewing time were strongly correlated with each other, and total work and force/chews also showed strong correlation (Fig. 2c). Duty cycle and fibre content were situated relatively far away in the PCA plot, and thus were not well correlated with any other mastication parameters.

3.4. Inter-individual variation in mastication and consistency of the participants

The participants were significantly different ($p < 0.05$) when jointly considering all mastication properties obtained from fifteen participants (Table 4l). There was a significant difference ($p < 0.05$) between participants for each chewing parameter except duty cycle for different products. The differences between the participants were more significant ($\eta^2 = 0.859–0.959$) in terms of total work, force/chew, chewing time and number of chews compared to EMG activity time and duty cycle ($\eta^2 = 0.309–0.749$). A PCA plot with high residual variance from the first two components (Fig. 2d) for all the variables indicates that the participants were not all consistent in explaining the mastication properties of puffs and flakes samples. There were 45% non-redundant residuals with absolute values >0.05.

Four extruded samples were significantly different ($p < 0.05$) when jointly considering chewing parameters (data obtained from fifteen participants) (Table 4d). Out of fifteen participants, 90% had significantly ($p < 0.05$) different total work, 67% had different chewing time and 60% had different numbers of chews and EMG activity time. Only 20% of participants had shown differences ($p < 0.05$) in force/chew, and the duty cycle was different for only one participant. Based on the above results it can be concluded that total work and chewing time were the most important parameters, followed by number of chews. More than 60% of participants were consistent in chewing/evaluating the extruded samples. In the PCA plot, since the individual residual variance from the first two components (Fig. 2e) for all the parameters was high, it may be concluded that not all participants were consistent in explaining the mastication properties of puffs and flakes samples. There were 40% non-redundant residuals with absolute values >0.05.

3.5. Bolus properties

Disintegration of the samples into particles was examined for the bolus samples ($n = 8$), grouped in three different chew types: six average chewers, one heavy and one light chewer (Fig. 3). A set of granulometric curves based on the data obtained from particle size measurements was produced to visualise the particle area distribution (Fig. 4). From the cumulative surface (%) vs particle area curve it is clear that puffs and flakes can be easily discriminated after mastication based on their particle size and particle area distribution. Visual examination of the photographs showed that puffs were masticated to smaller particles than flakes, and that RB addition increased the proportion of smaller particles (Figs. 4a-d). However, bolus samples with added RB also contained bigger particles than samples with 100% endosperm rye flour (ERF). These observations were confirmed by data on particle size distribution presented as granulometric curves showing the cumulative percentage of the total area occupied by particles (Fig. 4). Puffs and flakes with added RB had the highest share of small particles compared to corresponding products without RB. The presence of larger bolus particles in both flake samples was detected in the granulometric curves as a lower share of particles <100 mm^2 than in puffs. Both the removal of the smallest particles and the effect of swelling may have had an influence on the result. However, the effect of the removal of the smallest particles was the same for all the samples. As the analysis was carried out in water, the particles with less saliva were most probably swollen to some extent. Nevertheless, most of the differences in saliva uptake were not statistically significant. Mean particle area of the puffs (0.568 and 0.325 mm^2 with and without RB, respectively) and flakes (1.134 and 0.521 mm^2 with and without RB, respectively) varied between 0.325 and1.134 mm^2 and the average of all four puffs and flakes was 0.637 mm^2. Four extruded samples were significantly different ($p < 0.05$) when jointly (Table 4e) considering bolus properties and DF (data obtained from eight participant). The PCA plot shows that the

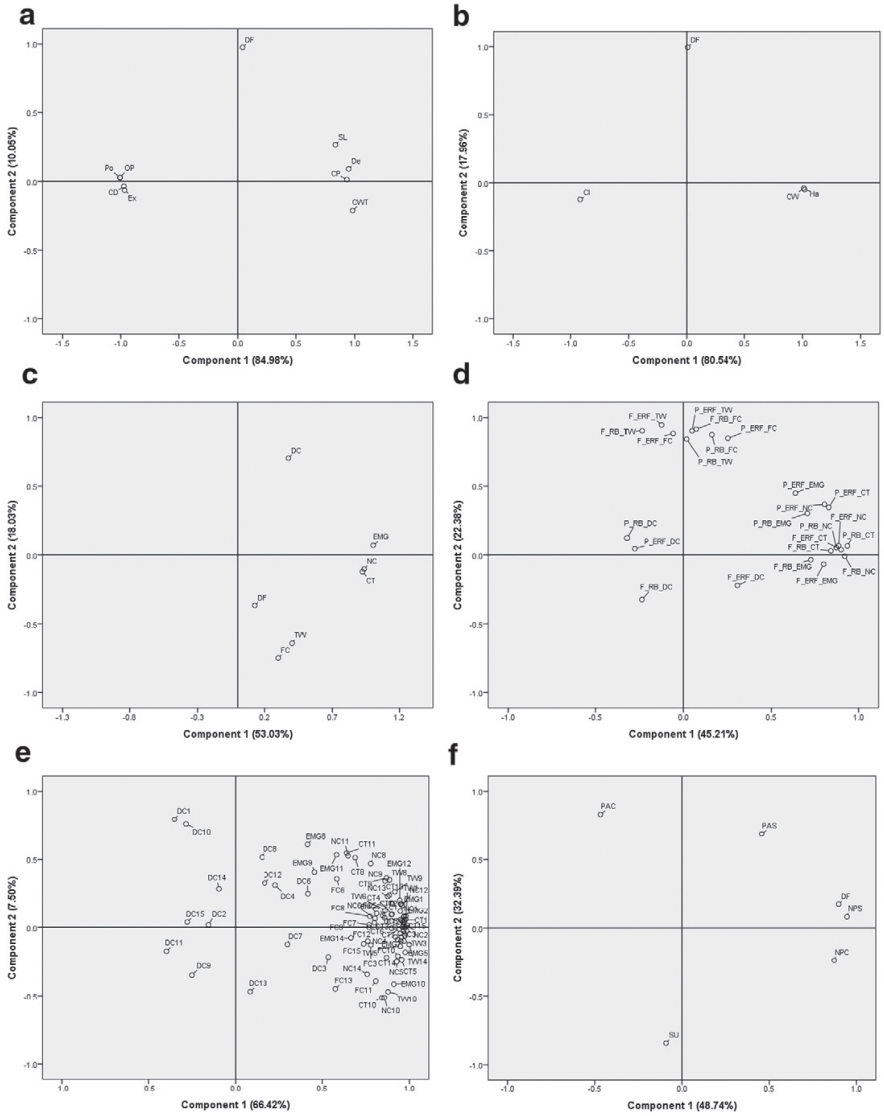

Fig. 2. PCA plots of **a)** macrostructural (Ex *expansion ratio*, De *piece density* and SL *specific length)* and microstructural properties (Po *total porosity*, OP *open porosity*, CP *closed porosity*) [KMO = 0.831 & Bartlett's $p = 0.000$], **b)** textural properties (Ha *hardness*, CW *crispiness work*, CI *crispiness index*) [KMO = 0.721 & Bartlett's $p = 0.000$], **c)** mastication properties (NC *number of chews*, CT *chewing time*, EMG *EMG activity time*, DC *duty cycle*, FC *force/chew*, TW *total work*) [KMO = 0.603 & Bartlett's $p = 0.000$], **d)** effects of mastication properties on puffs and flakes (P_ERF *100% rye flour puffs*, F_ERF *100% rye flour flakes*, P_RB *10% rye bran puffs* and F_RB *10% rye bran flakes* [KMO = 0.681 with Bartlett's $p = 0.000$], **e)** inter-individual variation of the participants 1–15 [due to the large number of variables the matrix was not a positive definite, and thus KMO and Bartlett's tests were not performed] and **f)** bolus properties (NPS *number of particles by sample*, NPC *number of particles by participants*, SL *saliva uptake*, PAS *particle area by sample* and PAC *particle area by participants*) [KMO = 0.658 & Bartlett's $p = 0.000$]. DF *total dietary fibre* and HI *in vitro hydrolysis index* of puffs and flakes made of 100% endosperm rye flour (ERF) and 90% ERF + 10% rye bran (RB).

total particle area ($p < 0.05$, $r = 0.566$) and the number of particles ($p < 0.05$, $r = 0.876$) were positively correlated with the fibre contents of the sample (Fig. 2f). Large inter-individual differences in bolus particle area of different participants masked the effect of the fibre effect. Peyron et al. (2011) found that at swallowing point, the mean particle size of cereal flakes was 1.52 mm. A higher number of chews (puffs < flakes) does not always result in a higher number of small particles, because of the loss of many small particles during intermediary swallows (Jalabert-Malbos et al., 2007).

3.6. Hydrolysis index of puffs and flakes

In the current study, extruded puffs and flakes had high hydrolysis indices (85.9%–94.5%) (Fig. 5). Previous studies also showed that extrudates had a high starch hydrolysis rate, regardless of their dietary fibre content (Anguita, Gasa, Martín-Orúe, & Pérez, 2006; Brennan, Merts, Monro, Woolnough, & Brennan, 2008). Both high shearing and high temperature profile of extrusion processing disrupts the structural integrity of starch granules and increases their susceptibility to

Table 3
Structural and textural properties of the extruded puffs and flakes.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Macrostructure				
Expansion rate (%)	570.67 ± 17.13 [d]	531.60 ± 24.64 [c]	106.88 ± 21.93 [b]	87.83 ± 9.67 [a]
Specific length (m/kg)	37.82 ± 1.72 [a]	40.07 ± 3.57 [a]	59.90 ± 7.92 [b]	71.44 ± 5.05 [c]
Piece density (kg/m^3)	115.41 ± 9.18 [a]	126.79 ± 18.83 [a]	2229.98 ± 580.40 [b]	2632.76 ± 430.46 [c]
Microstructure				
Porosity (%)	96.78 ± 0.44 [b]	96.48 ± 0.62 [b]	33.72 ± 4.65 [a]	36.66 ± 4.57 [a]
Open porosity (%)	96.78 ± 0.44 [b]	96.47 ± 0.62 [b]	33.72 ± 4.87 [a]	35.74 ± 4.90 [a]
Closed porosity (%)	0.01 ± 0.01 [a]	0.17 ± 0.22 [a]	1.48 ± 0.41 [b]	1.41 ± 0.52 [b]
Mean cell diameter (mm)	2.43 ± 0.25 [b]	2.31 ± 0.21 [b]	0.18 ± 0.03 [a]	0.17 ± 0.04 [a]
Mean cell wall thickness (mm)	0.12 ± 0.01 [a]	0.12 ± 0.01 [a]	0.17 ± 0.01 [c]	0.15 ± 0.01 [b]
Texture				
Hardness (N)	25.64 ± 0.26 [a]	29.73 ± 1.0 [b]	1868.02 ± 80.42 [c]	2107.10 ± 96.93 [d]
Crispiness work ($\times 10^{-2}$) (Nmm)	63.22 ± 6.33 [a]	73.35 ± 5.72 [b]	4266.64 ± 307.72 [c]	4954.43 ± 398 [d]
Crispiness index ($\times 10^{-7}$)	258,199 ± 12,635 [d]	184,155 ± 29,363 [c]	6.38 ± 1.02 [b]	4.21 ± 0.39 [a]

Values followed by **different letters** in the **same row** were significantly different ($p < 0.05$).

enzymatic breakdown, leading to an increase in the hydrolysis rate. In the current study, no difference in HI was observed between puffs and flakes made with 100% ERF. However, the HI of both puffs and flakes with added RB was significantly higher than that of puffs and flakes made with 100% ERF. RB addition increased the rate of starch digestion already in the early phase. After 30 min of enzymatic incubation, RB-enriched puffs (71.4 ± 1.5) and flakes (71.3 ± 2.8) had higher percentages of hydrolysed starch compared to the puffs (63.7 ± 2.2) and flakes (64.1 ± 2.9) without bran addition.

Insoluble DF content was increased by RB addition, which caused significant changes in the structural and textural properties of puffs and flakes. The HIs of rye puffs and flakes were significantly different ($p < 0.05$) when jointly considering expansion, hardness, piece density, porosity, crispiness index and DF (Table 4k). There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.957 and 0.997. DF content showed the strongest effect, followed by texture, macro- and microstructure, on the HI of the puffs and flakes. A strong positive correlation between DF and HI ($p < 0.05$, $r = 0.916$) indicates that increasing fibre content increased HI for puffs and flakes. The increased starch hydrolysis by bran addition might be associated with disruption in the starchy matrix due to the presence of insoluble bran particles. A similar phenomenon was earlier observed for bran-enriched pasta, in which higher *in vivo* glucose responses were observed after bran addition. The difference was concluded to result from the less dense structure of the pasta with bran addition, attributable to small fractures within the pasta matrix (Eelderink et al., 2015). There were clear structural and textural differences between flakes and puffs, which ultimately created differences in particle size (both in the bolus samples but also in the milled samples for the *in vitro* starch digestibility tests). After *in vivo* or *in vitro* mastication (simulated by milling), flakes had more fine particles compared to puffs, which further increased with bran addition. The presence of fine particles might have increased the availability of starch for digestive enzymes. *In vitro* starch HI of RB-enriched extrudates might be dependent on the concentration of the bran addition. In a recent study (Alam et al., 2016), we observed that when the concentration of RB decreased from 30% to 15%, there was a significant increase in the HI (69.0 vs 74.6). Addition of finely milled RB (D_{50} = 23.7 μm) might also have an effect on increased HI. Significant increase ($p < 0.05$) in the HI was observed in 15% fine (D_{50} = 28 μm) RB-enriched extruded puffs (74.6%) compared to puffs made of coarse (D_{50} = 440 μm) RB (70.3%) with a similar bran concentration (Alam et al., 2016). According to these results, the use of coarse bran at higher addition levels looks like a good strategy to decrease the HI. However, as it significantly interferes with the structural and textural properties, we recommend the use of fine bran to create palatable high fibre extrudates.

On the other hand, Brennan et al. (2008) observed slightly reduced starch hydrolysis of the extrudates (TDF: 15%) when either guar gum (soluble fibre) or wheat bran (insoluble fibre) were added as DF source. However, the hydrolysis of guar gum-enriched extrudates was significantly lower compared to the extrudates made of wheat bran. Considering the results obtained in the current study, but also based on existing

Table 4
Multivariate analysis of variance results of the studied properties.

	Wilk's Λ	Degree of freedom (hypothesis df, error df)	p	partial η^2
Effect on sample by				
a. Macro- and microstructure * DF	0.000006	F (27, 59) = 135.14	0.000	0.982
b. Texture * DF	0.000005	F (12, 66 = 550.13	0.000	0.983
c. Mastication properties * DF	0.293129	F (18, 145) = 4.37	0.000	0.336
d. Mastication properties (15 participants)	0.000001	F (24, 4) = 17.40	0.011	0.990
e. Bolus properties * DF (8 participants)	0.001760	F (12, 66) = 55.32	0.000	0.879
f. Macro- and microstructure * texture * mastication * DF * HI * bolus properties (8 participants)	0.000000	F (66, 22) = 151.76	0.000	0.998
Effect of DF on				
g. Macro- and microstructure	0.000362	F (32, 75) = 17.84	0.000	0.862
h. Texture	0.000709	F (12, 66) = 80.29	0.000	0.911
i. Mastication properties	0.317482	F (15, 143) = 4.95	0.000	0.318
j. Macro- and microstructure * texture * mastication * HI * bolus properties (8 participants)	0.000000	F (84, 30) = 23.70	0.000	0.984
Effect on HI by				
k. Expansion * hardness * piece density * porosity * crispiness index * DF	0.000004	F (66, 86) = 18.85	0.000	0.913
Effect on participants by				
l. Mastication properties (15 participants)	0.000000	F (336, 130) = 7.07	0.000	0.923

Strongest effect indicated by Wilks Λ value close to zero and partial η^2 value close to unity, $p < 0.05$.

Table 5
Mastication parameters of the samples.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Number of chews	17.7 ± 5.7 [a]	16.2 ± 5.50 [a]	29.2 ± 10.4 [b]	30.0 ± 7.4 [b]
Chewing time (s)	11.8 ± 4.2 [a]	10.7 ± 3.95 [a]	19.0 ± 6.7 [b]	19.9 ± 5.7 [b]
EMG activity time (s)	3.3 ± 1.1 [a]	3.0 ± 1.06 [a]	5.6 ± 2.6 [b]	5.6 ± 1.5 [b]
[1]Duty cycle	0.3 ± 0.04 [a]	0.3 ± 0.02 [a]	0.3 ± 0.03 [a]	0.3 ± 0.02 [a]
[2]Force/chew (%)	191.4 ± 68.6 [a]	193.6 ± 69.5 [a]	248.4 ± 74.0 [b]	254.5 ± 77.5 [b]
[2]Total work (%)	215.3 ± 118.6 [a]	193.3 ± 92.0 [a]	438.4 ± 175.7 [b]	473.4 ± 174.2 [b]
Saliva uptake (g/1 g puffs and flakes)	0.47 ± 0.10 [a]	0.45 ± 0.10 [a,b]	0.38 ± 0.07 [a,b]	0.37 ± 0.09 [b]
Moisture (%)	10.01 ± 0.01 [c]	10.02 ± 0.27 [c]	6.50 ± 0.01 [a]	7.49 ± 0.06 [b]

Values followed by the **different letters** in the **same row** were significantly different ($p < 0.05$).
[1] EMG activity time/chewing time.
[2] Normalized to the corresponding values of a reference product (white wheat bread).

literature, it is clear that the effect of dietary fibre addition on HI is dependent on various factors such as the fibre type, concentration, particle size and subsequent effects of the fibre on structure.

3.7. Interactions between structure, texture, mastication and bolus properties

Puffs and flakes were significantly different ($p < 0.05$) when all the studied properties were taken into account (Table 4f). All structural and textural properties and hydrolysis index were significantly different ($p < 0.05$) within the product, with a partial η^2 variation between 0.816 and 0.996. The differences between products were more significant in terms of hardness, piece density, expansion rate, porosity, number of particles by participants, number of particles by samples and DF content. In terms of mastication properties, the differences between the products were significant ($p < 0.05$) only for total work (data obtained from 8 participants).

Contrast results (K Matrix) showed that all ($p < 0.05$) variables except EMG activity time, duty cycle, force/chew, average particle area by participants and saliva uptake played a significant role in making the puffs and flakes different when 10% RB puffs were compared to 10% RB flakes. When the 100% ERF and 10% RB-enriched flakes were compared, there were no statistically significant differences in the mastication. Hardness and piece density dominated the product structure but there was no significant effect ($p > 0.05$) for crispiness index, expansion, porosity or cell diameter. However, all studied properties except duty cycle, work per chew and saliva uptake were jointly (Table 4j) and individually influenced by fibre content ($p < 0.05$).

The properties of puffs and flakes, representing hard and dense structure, respectively, were closely packed in the right side of the first component matrix, making a cluster, whereas the properties representing expanded and crispy structure were loaded in the left hand side of the first component matrix and closely packed together (Fig. 6). Mastication properties were weakly loaded in the first component matrix and formed a separate cluster. The number of particles by sample and by participants, hydrolysis index and total dietary fibre were loaded in the second component matrix. The component correlation matrix results with low coefficient value (= 0.098) indicated that the variables/parameters loaded in the first and second component were not correlated with each other. Mean particle area by participants

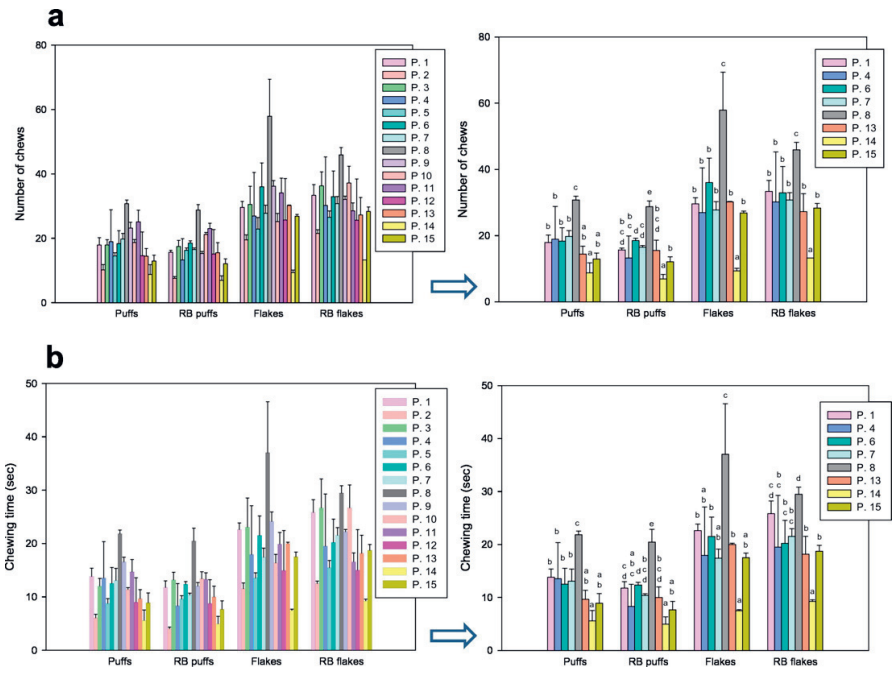

Fig. 3. Six average, one heavy and one light participants were chosen based on the mastication parameters of puffs and flakes chewed by 15 female participants: **a)** number of chews and **b)** chewing time. Data shown here are means of three replicates ± SD. The bars marked with different letters within each samples were significantly different ($p < 0.05$).

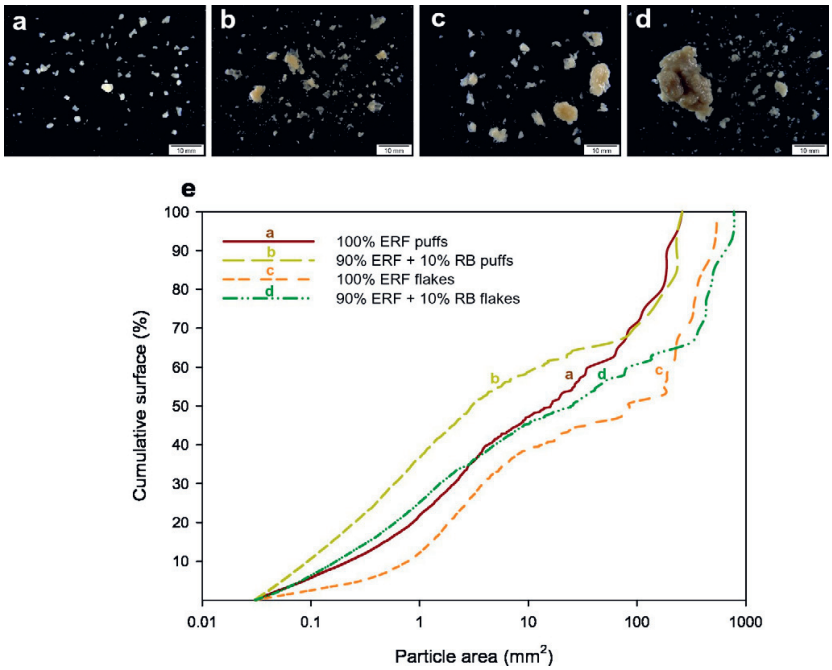

Fig. 4. Photographs of masticated samples of **a)** 100% ERF puff, **b)** 90% ERF + 10% RB puff, **c)** 100% ERF flake, **d)** 90% ERF + 10% RB flake (the white bar is 10 mm), and **e)** particle area distribution the same samples. The curves represent a cumulative percentage of the total area occupied by particles obtained from the boluses of 8 individual participants.

and duty cycle were weakly loaded in the plot due to the low loading value and high residual values.

Increase in cell diameter makes both products less hard and vice versa. Increased average cell diameter resulted in an increase in the C_i and decrease in the hardness and C_w of both extruded puffs and flakes, indicating that extrudates with lower hardness values were more expanded and crispier. We have previously reported that crispiness increased (high C_i and low C_w) with the increase of average cell diameter, indicating that extrudates with larger air cells were less hard and crispier (Alam et al., 2014). Increased crispiness with reduced hardness was also reported for bran-enriched oat extrudates in a previous study (Sibakov et al., 2015). High dietary fibre content in extruded snack products by RB addition has also earlier resulted in high hardness, low crispiness and poor microstructural properties of extruded snacks based on corn, wheat, rye and oat (Heiniö et al., 2016).

Flakes required more work for mastication and more force/chew than puffs, owing to their dense structure, low cell diameter and thick cell walls. The results are in line with a previous study in which bread with higher bulk density required higher total work and force/chew compared to lower density wheat bread matrices (Pentikäinen et al., 2014). Flakes had a hard, crunchy texture, whereas puffs had an easily broken, crispy texture. Food texture plays a major role in disintegration. Hard food products, in general, require longer mastication time and more chewing cycles for insalivation to produce a lubricated bolus (Jalabert-Malbos et al., 2007). Increased food hardness has earlier been shown to correlate positively with the number of masticatory cycles in different natural foods such as salted and roasted peanuts, raw and peeled carrots and raw and pickled gherkins (Jalabert-Malbos et al., 2007).

Rye bran addition increased hardness and decreased crispiness of both flakes and puffs. However, this variation observed in instrumental textural properties was not sufficient to induce changes in mastication behaviour. Jalabert-Malbos et al. (2007) reported that within different food categories (such as vegetable, meat, cheese and egg), product

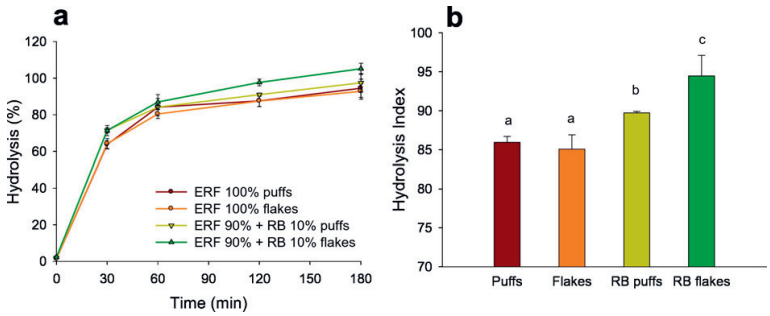

Fig. 5. a) *In vitro* starch hydrolysis (% of hydrolysed starch) of extruded puffs and flakes during 180 min incubation. **b)** Hydrolysis indices (HI = (A_{uc} of puffs or flakes / Average A_{uc} of white wheat bread) × 100) of puffs and flakes. Values are means of three replicates ± SD. The bars marked with different letters were significantly different ($p < 0.05$).

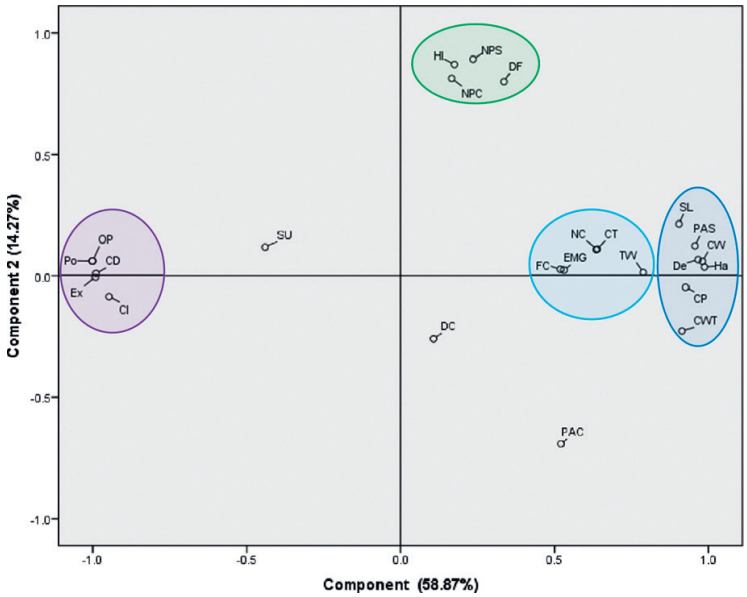

Fig. 6. PCA [KMO = 0.722 & Bartlett's $p = 0.000$] plot showing the correlation between macro- (Ex *expansion ratio*, De *piece density* and SL *specific length*) and micro- (Po *total porosity*, OP *open porosity*, CP *closed porosity*) structural and textural properties (Ha *hardness*, CW *crispiness work*, CI *crispiness index*), fibre content (DF *total dietary fibre*), *in vitro* hydrolysis (HI *hydrolysis index*) of extruded puffs and flakes and their mastication (NC *number of chews*, CT *chewing time*, EMG *EMG activity time*, DC *duty cycle*, FC *force/chew*, TW *total work*) and bolus properties (NPS *number of particles by sample*, NPC *number of particles by participants*, SL *saliva uptake*, PAS *particle area by sample* and PAC *particle area by participants*). Mastication and bolus properties were obtained from 8 individual participants.

texture had an effect on disintegration but the disintegration still varied depending on the individual participants. Fontijn-Tekamp et al. (2000) and Le Bleis, Chaunier, Montigaud, and Della Valle (2016) reported that extended chewing time had very little effect on particle size reduction but it rather allowed more saliva uptake for agglomeration of the fractured particle to form a suitable bolus. This is in agreement with our study, in which the flakes had a bigger share of large particles and a longer chewing time compared to the puffs. Therefore, both particle size reduction and saliva uptake are crucial during mastication.

Crispier and less hard extrudates disintegrated rather easily and produced more particles in the bolus (number of particle for puffs vs flakes: 718 vs 387/g of bolus sample; RB puffs vs RB flakes: 1406 vs 916/g of bolus sample). This led to increased total surface area of the disintegrated particles, which required more saliva for bolus formation and particle agglomeration. Saliva impregnation in masticated samples was on average 0.46 and 0.37 g saliva/g puffs and flakes, respectively. Inter-individual variation in saliva uptake was considerable, as was that in mastication pattern. Saliva uptake was negatively correlated (Fig. 6) with hardness ($p < 0.05$, $r = -0.422$) and positively with crispiness ($p < 0.05$, $r = -0.399$). A similar result was reported for crispy bread by Pangborn and Lundgren (1977), who reported that small particles of crisp bread required more saliva for lubrication and agglomeration to form a cohesive bolus which was easy to swallow.

In our study, it was noticeable that RB addition increased the number of smaller particles both for puffs and flakes. Reduction in starch content and increase in TDF content (Table 2) by RB addition made the matrices less porous and denser, resulting in a harder and less crispy product. Textural properties such as hardness have a significant influence on chewing and bolus formation. Chen, Khandelwal, Liu, and Funami (2013) observed that increased food hardness tends to result in smaller bolus particles, which is in line with our results within product categories. Boluses of relatively hard foods (e.g. nuts and cheese) were shown to have a smaller mean particle size compared to the boluses obtained from soft food (e.g. jelly and peach). It can be hypothesised that RB made the puffs and flakes less cohesive, and thus cluster formation of numerous small particles was less likely during bolus formation. The presence of smaller particles in RB-enriched puffs and flakes increased the susceptibility of starch to digestive enzymes and led to increased HI. Singh, Dartois, and Kaur (2010) reported in their review that increase in surface area by lowering the particle size distribution of the starch granule leads to a higher degree of starch hydrolysis.

The dominating factor in the variation of mastication process, particle size distribution and saliva uptake was inter-individual variability, e.g. some persons chewed for a longer period of time, and their bolus samples had uniform small particles, whereas others chewed for shorter periods of time and were ready to swallow already when some particles were still significantly large and uneven in shape. In earlier studies a wide variation between individuals has also been reported for mastication parameters and particle size distribution (Foster, Woda, & Peyron, 2006; Jalabert-Malbos et al., 2007). Le Bleis et al. (2016) reported that particle size of extrudate bolus before swallowing varied between 0.7 and 2.66 mm due to inter-individual differences. Therefore, inclusion of a higher number of participants in this study would possibly have led to the detection of small differences in the mastication patterns due to minor structural differences between samples within each sample type.

4. Conclusions

Puffs and flakes were notably different in their structure and texture and the differences were reflected in their mastication properties. Puffs with less hard and crispy structure degraded to smaller particles in oral processing and required less work for bolus formation than flakes, but the wide inter-subject variation in mastication prevented the detection of the effects of such subtle structural adjustment on mastication properties. Bran addition resulted in increased hardness and reduced crispiness for both puffs and flakes, but significant increase in density was observed only for flakes. Hardness and density dominated the

mastication properties, rather than crispiness. The harder puffs or flakes required more mastication. In contrast, when the product become expanded and crispy it required less mastication. Despite the striking differences in structure and texture, the hydrolysis index of puffs and flakes did not differ. However, addition of rye bran increased the number of smaller particles in the bolus as well as the hydrolysis rate, which was noticeable in the early phase of digestion at 30 min, indicating that the disintegration process and consequently the particle size of the bolus had a significant role on the starch digestion rate. Therefore, a small increase in fibre content in an extruded matrix may not always reduce HI but, as seen in these data, the effect may even be opposite.

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PUBLICATION IV

The effect of structure and texture on the breakdown pattern during mastication and impacts on *in vitro* starch digestibility of high fibre rye extrudates

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The effect of structure and texture on the breakdown pattern during mastication and impacts on *in vitro* starch digestibility of high fibre rye extrudates

Syed Ariful Alam, *[a,b] Saara Pentikäinen,[a] Johanna Närväinen,[a] Kati Katina,[b] Kaisa Poutanen[a] and Nesli Sozer[a]

The snack product category is lacking palatable, high dietary fiber containing products. This study explored how the addition of native or fermented rye bran influences the texture and sensory properties of endosperm rye flour based extrudates. In addition, mastication and bolus properties (n = 26), and *in vitro* starch digestibility were assessed. Three high fiber extrudates based on endosperm rye flour (EF) were produced with addition of either 40% native rye bran (NBE) or 40% fermented rye bran (FBE), and with no added bran (EFE) to achieve two pairs of extrudates to compare. EFE and FBE had different composition but resembled each other regarding macrostructure and the second pair (NBE *vs.* FBE) had similar core composition but different structure due to bran fermentation. The fermentation of bran was performed using exopolysaccharide (EPS)-producing strain *Weissella confusa*, which led to 3% (3 g per 100 g bran; dry weight) *in situ* dextran production. The compositionally similar extrudates (NBE *vs.* FBE) varied in both structure and instrumental texture: FBE were less dense, less hard and crispier than NBE. The extrudates with different composition (EFE *vs.* FBE) varied regarding instrumental texture: FBE were less hard and crispier than EFE. There were also subtle structural differences FBE being somewhat denser than EFE. NBE and FBE differed regarding sensory texture while textures of EFE and FBE were perceived similar. Mastication properties of the different products did not exhibit remarkable differences. There was a large number of smaller particles in both NBE and FBE bolus samples. The fragile structure of FBE, and its lower bolus viscosity, led to high *in vitro* starch digestibility. The results demonstrate that the structural attributes of the extrudates, rather than the core composition, dictate the breakdown pattern during mastication and *in vitro* starch digestibility. The extrudates with similar composition may be digested at different rates depending on their structural attributes. Although FBE had higher *in vitro* starch digestibility, its high DF content, palatable texture and improved sensory properties were important determinants underlying eating quality and therefore it could be a promising product to snack food category.

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Introduction

Rye (*Secale cereale* L.) is a widely cultivated cereal grain in Northern and Eastern Europe. Rye has the highest content of dietary fiber (DF) among cereal grains, and is typically consumed as wholegrain including the DF rich bran (39–48% DF, mostly insoluble). Rye bran is also rich in starch (13–28%), protein (14–18%), pentosans (23%) and β-glucan (3.5–5.3%).[1–3] Rye bran could be utilized as a raw material in the manufacture of DF- and protein-enriched extruded products. Addition of wheat bran, guar gum and inulin into extruded products at DF levels of 5–15%,[4] and of 15% fenugreek polysaccharide into chickpea and rice blend,[5] reduced both *in vitro* starch digestibility rate and *in vivo* glycaemic responses. A similar effect has been shown (*in vitro*) by adding 30% rye bran into rye flour based extrudates.[6] However, bran addition interfered with the palatability of extrudates *via* reduced expansion and crispiness and increased hardness, limiting its use particularly at addition levels above 5% of flour.[6–9]

Processability of wheat and rye bran was improved by fermentation with lactic acid bacteria (LAB) and yeast. Fermentation-induced modification of wheat and rye bran has

[a]*VTT Technical Research Centre of Finland Ltd, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland. E-mail: ariful.alam@vtt.fi; Tel: +358 40 578 9984*
[b]*Department of Food and Nutrition, Agnes Sjöbergin katu 2, P.O. Box 66, 00014 University of Helsinki, Helsinki, Finland*

been shown to improve microstructure, texture, flavor and shelf-life of high DF breads.[10–12] Many LAB belonging to the species *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* are capable of producing an inducible dextransucrase enzyme, which converts sucrose ($nC_{12}H_{22}O_{11}$) to dextran ($(C_6H_{10}O_5)n$ and fructose ($nC_6H_{12}O_6$).[13–15] Dextrans are a large group of α glucans, in which the main polymeric backbone chain consists of α-(1 → 6)-glycosidic linkages (>50%) with α-(1 → 2)-, α-(1 → 3)-, and/or α-(1 → 4)-branched linkages.[15–18] *Weissella confusa* is reported to be efficient in the production of linear dextrans with molecular weights around 1.8×10^7 g mol^{-1}, with mainly 97% α-(1 → 6) and a very few (3%) α-(1 → 3)-linkages.[14,19–21] Dextran produced during *in situ* fermentation has been shown to have technological benefits over externally added dextran.[22] Addition of 20% rye bran into rye flour-based extrudates with 5% commercial dextran did not improve the structural properties compared to untreated polished bran extrudates.[22] Polysaccharides produced during *in situ* fermentation were also effective in improving bread structure compared to externally added polysaccharides.[23]

Although fermentation of rye bran has been shown to have technological benefits in baking, its applicability has not been studied widely in extruded products. In a previous study[22] rye bran fermentation with EPS-producing *Weissella confusa* improved the structural and textural properties of extrudates, although the effects on mastication, *in vitro* starch digestibility and product viscosity were not investigated. During mastication of food, contraction of the jaw muscles occur and a bioelectrical signal propagates to the adjacent muscle cells, to help to coordinate muscle contraction. This bioelectrical signal could be measured by using Electromyography (EMG), which is a commonly used technique in studying the relationships between oral processing and food texture.[24] Food structure and texture have a prominent role in mastication. Mastication is the first step of digestion, during which food is broken down to small particles and mixed with saliva, preparing the bolus for swallowing. The size of particles in the bolus varies depending on food structure and texture.[25,26] The rate of food breakdown is inversely related to hardness.[27] Hard and dense extruded products required more intense mastication than crispy and expanded extrudates.[28,29] The proportion of small particles in the bolus increased as a result of bran addition, which further increased *in vitro* starch digestibility.[28] However, further research is needed to understand the mechanisms and factors explaining the structure of cereal solid foams and their disintegration in the mouth and during digestion.

The aim of this study was to evaluate the effect of structure, texture and process-related factors (*e.g.* bran modification) in oral processing, bolus formation and *in vitro* starch digestibility of rye extrudates with varying structures and compositions. Therefore, two different pairs of extrudates were prepared; the first pair had similar macrostructure and expansion but different composition (EFE *vs.* FBE), and the second pair had similar core composition but varied in terms of structure and texture (NBE *vs.* FBE). The targeted variation of these properties is illustrated in Fig. 1.

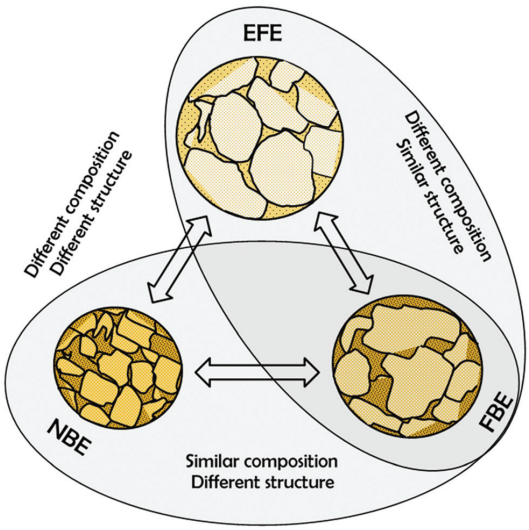

Fig. 1 Schematic overview showing the structural differences between the extrudates made of 100% endosperm rye flour (EFE), 40% native rye bran (NBE) and 40% EPS fermented rye bran (FBE).

2 Materials and methods

2.1. Raw materials

Endosperm rye flour (EF) was obtained from Helsingin Mylly (Järvenpää, Finland). Rye bran with high DF content was prepared by milling and air classification of native rye kernels (Jalon Mylly, Kouvola, Finland) with a protocol published by Nikinmaa *et al.*[22,30] Native rye kernels were milled by 100 UPZ-lb fine impact mill (Hosokawa Alpine AG, Germany) using a stainless steel pin discs (17 800 rpm), followed by air classification (speed 3500 rpm, airflow 220 m^3 h^{-1}, feed rate 50 kg h^{-1}) (British Rema Minisplit, Chesterfield, UK). Repeated milling and air classification was performed in order to produce a coarse fraction (34%) of the material. After the second air classification the coarse fraction (23%) was collected for extrusion and termed as native bran (NB).

2.1.2. Bioprocessing of bran. Bioprocessing of native bran was performed to obtain fermented bran (FB) by using EPS producing *Weissella confusa* (VTT E-133279) from the VTT Culture Collection (VTT Technical Research Centre of Finland, Espoo) using a protocol published by Nikinmaa *et al.*[22] Starter cultures of *Weissella confusa* were prepared revitalizing in MRS (de Man, Rogosa and Sharpe) broth (Oxoid LTD, Basingstoke, Hampshire, United Kingdom), before subculturing in GEM (general edible medium, containing 2 g per 100 ml glucose and sucrose, 3 g per 100 ml soy peptone, 0.7 g per 100 ml yeast extract, 0.1 g per 100 ml $MgSO_4 \cdot 7H_20$ in 0.01 mol l^{-1} pH 6.3 potassium phosphate buffer). Fermentation was performed with a bran : water ratio of 22 : 78. Sucrose was used as a substrate for dextran production thus 10 g per 100 g of the bran was replaced with sucrose. Sucrose was dissolved in water

(25 °C) in a large glass beaker and cell suspension was added and mixed together. The native bran was added later in the mixture and carefully mixed to avoid lump formation (no further mixing during fermentation). The beaker was then covered with aluminum foil and incubated at constant temperature of 25 °C for 20 hours. Fermentation of bran sample were performed in duplicate.

2.1.3. Chemical analysis of bran. Acidification of bran, before and after fermentation were carried out as described by Kajala *et al.*[30] Samples for pH and Titratable Acidity (TTA) analysis were taken at the beginning and the end of fermentation and were performed in duplicate. The values of pH were determined on-line by a pH meter (Model HI 99161, Hanna Instruments, Woonsocket, RI, USA) using a food penetration probe. Samples for final pH and TTA analysis were collected at 20 hours of fermentation. Final pH and TTA was determined by adding 10 g of fermented bran in 100 ml of distilled water and was titrated with 0.1 M NaOH using an automatic titrator (EasyPlus Titrator, Mettler-Toledo, Schwerzenbach, Switzerland). The TTA was expressed as the amount of NaOH used (ml) for titration.

2.1.4. Microbiological analysis of bran. The microbiological analyses were carried out of the bran material (in duplicate) at the beginning and at the end of fermentation as described by Kajala *et al.*[30] Bran samples (10 g) were homogenized with sterile saline (90 ml) in a Stomacher 400 lab blender (Seward Medical, London). Serial dilutions were made and the enumeration of LAB was carried out by plating on MRS (de Man, Rogosa and Sharpe) agar. From plate count agar (PCA, Difco Laboratories, Detroit, MI, USA), aerobic heterotrophic bacteria were determined. Cycloheximide (0.001%) was added to the PCA plates to prevent fungal growth. A yeast mold (YM) agar (Difco Laboratories) was used to determine the growth of yeasts and molds. To prevent bacterial growth, 0.01% of chloramphenicol and chlortetracycline were added to YM agar and to limit the spreading of fungal colonies, 0.02% of Triton-X 100 was used. The plates for MRS agar was incubated anaerobically at 30 °C for 2–3 days and PCA and YM agars were incubated at 25 °C for 2–3 days. After fermentation, fermented bran sample were dried in a Christ Epsilon 2–25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and ground using 100 UPZ-lb Fine impact mill (Hosokawa Alpine AG, Germany) with stainless steel pin discs (17 800 rpm).

2.1.5. Dextran content analysis. Dextran content in fermented bran was analysed by an enzyme-assisted method as described by Kajala *et al.*[30] Dextran was measured from the duplicate bran fermentations and also for the control native bran. A high-performance anion-exchange chromatography coupled with pulsed amperometric detector (HPAEC-PAD) was used to quantify the glucose formed after enzyme hydrolysis. The amount of dextran formed was estimated as the sum of anhydroglucose and was corrected with a recovery factor of 1.5. The recovery factor was determined with preliminary experiments as described by Katina *et al.*,[31] to take into account the recovery of dextran in bran matrices. The dextran yields were calculated using eqn (1) and all amounts are given as percentages of dry weight (% d.w.). The amount of anhydroglucose in sucrose was calculated by multiplying with (0.4737), which is the molar mass ratio of anhydroglucose to sucrose.

$$\text{Dextran yeilds} = \frac{\text{FD}}{(\text{IS} - \text{FS}) \times 0.4737} \quad (1)$$

where IS is the initial sucrose (endogenous sucrose in the raw material + added sucrose), FS is the final sucrose after fermentation and FD is the final dextran. Dextran contents (in %) of the FB and control NB was 2.96 ± 0.13 (dry weight) and 0.84 ± 0.06 (dry weight). Native rye bran has some sucrose in itself. Therefore, there is some dextran formed also in the control bran.

2.1.3. Particle size analysis of the bran material. Particle size of the bran material (NB and FB) was analysed with a Beckman Coulter LS 230 (Beckman Coulter Inc., CA, USA) using liquid module and with ethanol as a carrier as described by Alam *et al.*[32] Milling of rye bran was optimized in order to obtain similar particle size distributions of NB and FB (median particle sizes were: D_{50} = 361 ± 49 μm). Particle size analysis was performed in duplicate.

2.2. Extrusion processing

Feed materials for extrusions were prepared by adding 40% FB or 40% NB into EF in order to obtain the high fiber extrudates, while 100% EF was used to obtain extrudates for reference. The extrusion processing was performed using the processing parameters described by Nikinmaa *et al.*[22] Small amount of salt (0.8% of the total mass of all recipes) was added in the bran-flour mixture. Extrudates were prepared using a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) with a constant screw speed of 350 rpm, feed rate of 60 g min^{-1} and a temperature profile increasing gradually from 80 to 120 °C from feed inlet to die exit in the following order: 80–95–110–120 °C (sections 1 to 4). A co-rotating twin screw feeder (K-Tron Soder, Niederlenz, Switzerland) was used for feeding of the flour-bran mix. Water (2.0–3.5 g min^{-1}) was injected into the section 1 of the extruder barrel in order to obtain the desired moisture contents in the extrudates. A pair of twin screws and a 3 mm die were used in all extrusion trials. Feed rate calibration was performed for each recipe prior to extrusion. During extrusion, extrudates were collected from the die exit and dried in an oven for 30 min at 100 °C. Extrusion processing for each recipes was performed in duplicate. Torque values were recorded during extrusion (variation was between 70 and 80%), and the specific mechanical energy (SME)[27] was calculated using eqn (2):

$$\text{SME}(\text{kW h/kg}) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (2)$$

where ω is the screw speed (rpm), ω_r is the maximum screw speed of the extruder used (500 rpm), τ is the torque (%), Zr is the maximum power capacity of the extruder (2 kW) and Q is the feed rate (kg h^{-1}). The calculated SME values were varied between 0.27–0.31 kW h kg^{-1}.

2.3. Compositional analysis of the extrudates

Extruded samples were analysed for total starch content by AACC method no. 76.13,[33] insoluble and soluble dietary fiber by AOAC method no. 991.43,[34] total protein content by AACC method no. 46-11A[35] and fat by AOAC method no. 922.06.[36] Moisture content of the extrudates was determined by drying the samples in an oven at 130 °C for 2 hours. All the analysis were performed in triplicate and result reported as mean ± SD of three replicates.

2.4. Properties of the extrudates

2.4.1. Macrostructural analyses. The measurements of expansion rate, specific length and piece density were made from 30 replicates (15 from each extrusion batch) of each extrusion recipe using the method described by Alam *et al.*[32] A Vernier calliper was used to obtain the length and diameter of extrudate samples. The diameter was measured at three different points of an extrudate ribbon (9.7–10.5 cm long) and the average value was obtained, which represented the diameter of each sample.

Expansion rate was calculated with eqn (3):

$$\text{Expansion rate}\,(\%) = \frac{D_e}{D_d} \times 100\% \quad (3)$$

where D_e is the diameter (mm) and D_d is the diameter of the die (3 mm).

Specific length was calculated with eqn (4):

$$\text{Specific length}(\text{m/kg}) = \frac{L_e}{m_e} \quad (4)$$

where L_e is the length of the sample (m) and m_e is the mass of the sample (kg).

Piece density was calculated with eqn (5):

$$\text{Piece density}(\text{kg/m}^3) = \frac{4 \times m_e}{\pi \times (D_e)^2 \times L_e} \quad (5)$$

where m_e is the mass of the sample (kg), D_e is the diameter sample (m) and L_e is the length of the sample (m).

2.4.2. Textural properties analyses. A uniaxial compression test was used to determine the instrumental texture of the extrudates using a TA.XT2 Texture analyser (Stable Micro System Ltd, Godalming, United Kingdom) equipped with a 30 kg load cell and 25 mm diameter cylinder aluminium probe. Texture analysis samples were prepared by cutting the extrudate ribbon to 10 mm length (10.31–18.05 mm diameter) and placed vertically between the texture analyser platform and the aluminium probe. The samples were deformed at 70% strain with a test speed of 1 mm s^{-1} and with an acquisition rate of 200 points per s. Trigger force was set to 5 g and trigger type was selected to 'Auto (Force)' in the settings window. The force–displacement (f–d) curves were obtained (Fig. 2) in order to assess the textural properties of the extrudate samples. The force (in y-axis) and time (in x-axis) threshold was 0.049 N and 1 s, respectively, and was remain constant for all TA analysis. The analysis was performed with 80 replicates (40 from each extrusion batch) of each extrusion recipe using the method described by Alam *et al.*[32] In f–d curve, the number of peaks represents the number of cell wall ruptures during compression and hardness (F_{max}) is the maximum force needed to initiate cell wall crack. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) and a predefined macro was used to obtain the values of hardness (F_{max}), crispiness work (C_w) and crispiness index (C_i). The calculation was performed using the formulas published by Alam *et al.*[32] High C_i and low C_w values indicate high crispiness, whereas low C_i and high C_w values indicate low crispiness of the sample.

Crispiness work (C_w) was calculated with eqn (6) (Van Hecke *et al.*[37])

$$C_w(N\,\text{mm}) = \frac{A}{N} \quad (6)$$

where A is the area under the f–d curve (Nmm) and N is the number of peaks.

Crispiness index (C_i) was calculated with eqn (7) (Heidenreich *et al.*[38])

$$C_i = \frac{L_N}{A \times F_{mean}} \quad (7)$$

where L_N is the normalized curve length (length of actual curve/F_{max}), A is the area under the f–d curve (Nmm) and F_{mean} is the sum of the actual force values in the data file divided by the number of data points (N).

2.4.3. Sensory analysis. A trained sensory panel (n = 12) of VTT Technical Research Centre of Finland profiled the sensory attributes of the extrudates. Members of the sensory panel have passed the basic colour vision test, odour test and taste test and they have been trained for sensory profiling method. A training session was organized where the panellists familiarized themselves with the products and the key attributes relevant to the product category were defined. The defined sensory attributes were hardness, crispiness, coarseness, thickness, sliminess and intensity of overall flavour. Descriptive sensory profile analysis was conducted.[39] All descriptors were verbally anchored and reference samples were used for most attributes to define the extremes. Sensory attributes were evaluated using a 10 cm line scale anchored from "not at all = 0" to "extremely = 10". During the evaluation sessions the lightning was adjusted to hide the colour of the products. Samples were offered as 2 cm pieces. The panellists were instructed on how to treat the sample during the evaluation (for example "Chew the sample using your back teeth until the sample is ready to be swallowed and assess the properties") (Table 1). Sensory profiling of the samples was conducted in duplicate sessions in two consecutive days by all the panellists. The presentation order of the samples was randomized within each test day and the samples were blind-coded by 3-digit numbers. Water was served for cleaning the palate between different samples. During the session the scores were collected and recorded using software (Compusense Five, Ver 5.4.15, CSA Computerized Sensory Analysis System, Compusense Inc., Guelph, ON, Canada).

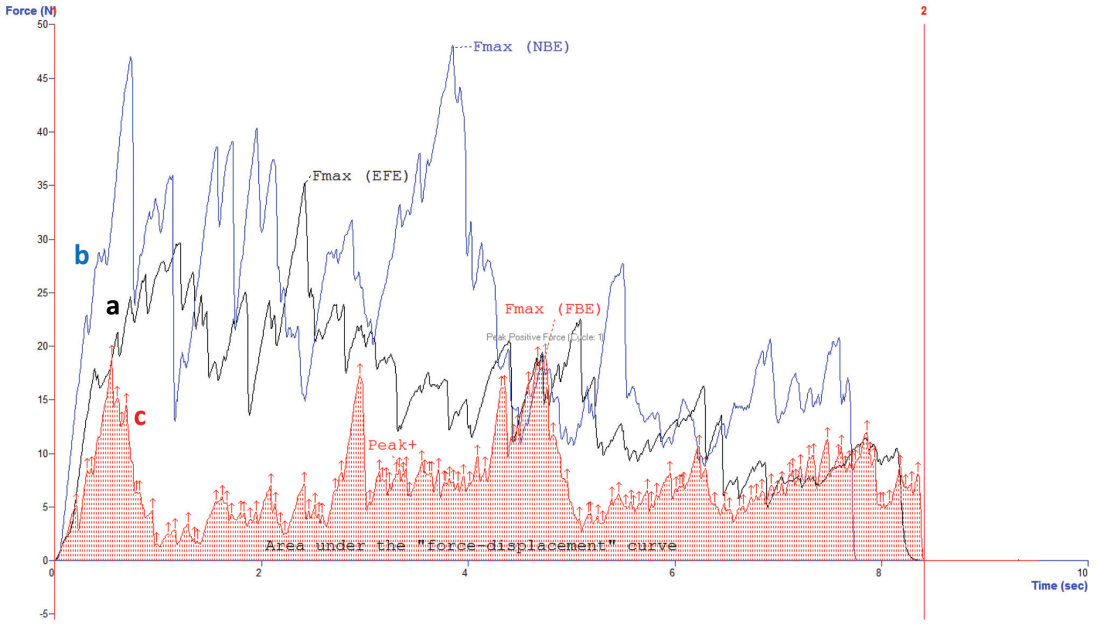

Fig. 2 Representative "force–displacement" curves obtained from (a) EFE, (b) NBE and (c) FBE to assess the textural characteristics.

Table 1 Definition of the sensory attributes and instruction given to the sensory panel (n = 12)

Properties	Instruction to sensory panel	Reference product
Crispiness	"Place the sample vertically between molars and evaluate crispiness from the first bite" Breaking sound (first bite) of a crispy product is sharp, and clear. The sound of a crispy product fades fast during the first bites. Crispy product disintegrate into fine particles fast	Rye flakes are not crispy (= 0) Baby snacks are crispy (= 10)
Hardness	"Place the sample vertically between molars and break the sample down with your molars" The more force need to break down the sample the harder it is	Baby snacks are not hard (= 0) Rye flakes are hard (= 10)
Coarseness	"Chew the sample until swallowing point and press the bolus (masticated sample) with tongue against palate" Large particle between tongue and the palate indicate coarseness	Baby snacks are not coarse (= 0) Rye flakes are coarse (= 10)
Thickness	"Chew the sample until swallowing point and evaluate the thickness of the bolus"	Berry juice is not thick (= 0) Rye smoothie is thick (= 10)
Sliminess	"Chew the sample until swallowing point and evaluate the sliminess of the bolus (mixture of the sample and saliva)"	Berry juice is not slimy (= 0) Viili (processed sour whole milk from Finland, similar to yoghurt) is slimy (= 10)
Intensity of overall flavour	"Chew the sample and hold it in your mouth and evaluate the intensity of overall flavour".	

2.4.4. Viscosity. The viscosity of the ground extrudate sample was analysed using a Rapid Visco Analyzer (RVA-Super4, Newport Scientific, Warriewood, Australia). Extrudate samples were ground using a Retsch mill at 6000 rpm speed with a 1 mm sieve. For each experiment, about 5 g of sample was dispersed into 25 ml of distilled water in order to obtain a homogeneous and viscous slurry. Experiment time was set for 180 min with initial stirring speed was 960 rpm (10 s) and later constant stirring speed of 120 rpm. A constant temperature (37 °C) was used during the experiment. Viscosity by RVA depend very strongly on the dry matter concentration. Slight changes in the amount of water or sample may introduce error in the final viscosity. Therefore, 14% (commonly used % moisture in standard RVA protocol) water was kept constant for all samples. For this purpose, the amount of distilled water and sample was calculated prior to experiment using pre-installed calculator with Thermocline software (TCW3). The adjusted weight of the sample (4.63–4.71 g) and water (25.29–25.34 g) was varied depending on their moisture contents (7.11–8.61%) of the extrudates. Weight of the sample

and water was determined by analytical balance with 0.001 accuracy. The water was weighed in an empty canister and the carefully weighed ground sample was slowly dispersed in the water. To avoid lump formation, RVA paddle was pumped back and forth for 10 times before placing the canister into the RVA equipment. The pH of the distilled water was adjusted to 6.9 (either by 0.1 mol l^{-1} NaOH or HCl) before performing the experiment. Thermocline software (TCW3) was used to obtain the final viscosity (cP) after cooling to 25 °C. The measurements were performed in triplicate and the results were reported as mean ± SD.

2.4.5. Hydrolysis index. Starch hydrolysis index (HI) of the extrudate samples was determined using a protocol published by Sozer *et al.*[40] and Alam *et al.*[28] Extrudate samples were ground (Retsch Ultra Centrifuze Mill ZM 200, 6000 rpm, 1 mm sieve) to mimic the particle size after mastication (visually observed). Ground extruded samples of about 1.4–2.0 g (to get 1 g starch in the sample) were taken in a beaker and same amount of distilled water (1 : 1) was added. A 100 ml 0.05 mol l^{-1} sodium potassium phosphate buffer ($KH_2PO_4{\cdot}Na_2HPO_4{\cdot}2H_2O$, pH = 6.9) was added in the beaker and pH of the solution was adjusted to 6.9 with 0.1 mol l^{-1} NaOH or HCl. Two different buffer addition methods (either after 15 minutes soaking or directly after adding water) were tested. The latter method was chosen to avoid lump formation. The solution was then incubated for 180 min at 37 °C with porcine pancreatic (110 U, 1 ml per 1 g starch) α-amylase (A6255-25MG, DFP Treated, Type I-A, saline suspension, ≥units per mg protein, 0.59 ml, 42 mg protein per ml (UV), 1151 units per mg protein, Sigma-Aldrich Co. LLC, USA). Samples were taken every 30 min for maltose analysis. After 180 min incubation with porcine pancreatic α-amylase, the solution was uniform and no lump was observed. The area under the curve (A_{uc}) was calculated using Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA, USA) using the preloaded macro. Incremental A_{uc} was calculated using the formula of trapezoidal area at 0 min = $\frac{1}{2} \times (abs_0 + abs_{30}) \times (t_{30} - t_0)$ followed by 30, 60, 120 and 180 min (here, abs = mg of maltose per 1 g soluble starch). HI values were calculated for each time interval (0, 30, 60, 120 and 180 min) and the results were reported as mean value of three replicates. Air dried white wheat bread was ground and was used as a reference product. HIs of extruded samples were calculated by comparing the areas under the curve to that of wheat bread (average of three analyses) using the formula: HI = (A_{uc} of extrudates/Average Auc of white wheat bread) × 100. The HI analyses were performed in triplicate and the results were reported as means ± SD.

2.5. Mastication trial

2.5.1. Participants. Participants (n = 26) for the mastication trial with electromyography (EMG) measurements were enrolled through an email and public advertisement near the study location. The eligibility of the participants was checked through screening questionnaire. The eligibility criteria included women aged between 20 and 40 years, BMI between 18.5 and 25 kg m^{-2}, with stable body weight (±4 kg during the previous year) and a habit of eating breakfast. Persons with missing teeth (except 3rd molars) or with diagnosed acute temporomandibular disorders (self-reported), persons with abnormal eating behavior (according to the Eating Disorder Diagnostic Scale; Stice *et al.*[41]), pregnant or lactating women, and persons with dietary restrictions (*e.g.*, celiac disease, allergies or aversions to cereal foods or high carbohydrate foods) and smokers were excluded. Young healthy females were recruited to avoid the possible variation in mastication pattern. The interested participants who fulfilled the inclusion criteria were invited to an information visit. The participants who decided to participate in mastication trial, signed an informed consent form. In total 26 female participants were included in mastication trial and were conducted during October–December 2015. Characteristics of the female participants are described in Table 2. To compensate time and effort, one movie ticket per study visit was offered to all the participants. The coordinating Research Ethics Committee of the Helsinki and Uusimaa Hospital District has approved the study protocol. The mastication trial was conducted according to the ethical principles of good research and clinical practice as described in the declaration of Helsinki. The trials were registered in ClinicalTrials.gov (NCT02554162).

Table 2 Characteristics of the participants for mastication trial (n = 26)

	Mastication trial (n = 26)	
	Avg. ± SD	Range
Age	31.7 ± 7.5	19–50
BMI	22.2 ± 1.9	19.1–27.3
Eating behaviour[a]		
Cognitive restraint	45.7 ± 16.6	11–72
Uncontrolled eating	27.6 ± 10.3	11–48
Emotional eating	33.3 ± 24.7	0–89

[a] 18-Item three-factor eating questionnaire (Karlsson *et al.*[42]) was used to measure eating behaviour.

2.5.2. Procedure. Mastication trials for electromyography (EMG) measurements of extrudate products were performed using a protocol published by Pentikäinen *et al.*[29] All trials were conducted using a crossover and single-blind design, in which the participants attended one study visit and masticated three extrudate products in random order. All extrudate products were masticated in three replicates. Participants were instructed to eat breakfast 1–1.5 h before the study visit, which was scheduled 8–11 in the morning. Participants were familiarised with the study procedure by going through the protocol with bread samples before the actual mastication trial. The coded extrudate samples were served in a random order and three portions of each extrudate sample were masticated in a row. Each portion included two pieces (2 cm × 1–1.8 cm) of extrudate samples (1 g). The participants were instructed to masticate each portion of sample until they feel the portion is ready to swallow and then expectorate the bolus in a plastic cup. There was a break between different extrudate products,

during which the mouth was rinsed with water. After completion of the mastication of all the study products, the participant was offered three pieces of chewing gum and was asked to chew each for 20 s. During the mastication trials, oral processing was characterised by measuring the electrical activity of facial muscles using electromyography. Although the force generated by four muscle was linearly relative to the measured voltage, the calibration varies between different subjects. Therefore, individual data on oral processing of chewing gum[29] was used as a reference for force parameter, to get an indication of the relative force needed to masticate each of the samples. All the mastication sessions were video recorded in order to support the data analyses.

2.5.3 Mastication properties with electromyography (EMG). Electrical activities of masticatory (masseter and temporal) muscles during mastication were measured with an EMG equipment (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. Masseter and temporal muscles were identified by touching when the participant gritted her teeth. The skin was cleaned with 70% ethanol and bipolar electrodes were placed on the muscles of both sides of the face. A reference electrode was placed on the cervical vertebra. EMG activity was measured and recorded continuously throughout the mastication session. In the EMG acquisition system, the data blocks for each chewing period were isolated for analysis using markers added to the EMG data. Experiment minute records and the video recording of each mastication session were kept to support the data analysis. The elimination of high frequencies and background fluctuations of the EMG time series was performed by applying chemometric techniques on the extracted onset, duration and amplitude of each chewing event.[43] Chewing force and work parameters were normalized to mastication data obtained from chewing gum trial. A Matlab® (The MathWorks Inc., Natick, MA, USA) software was used to analyze EMG data. The calculation of EMG activity time, chewing time, duty cycle (EMG activity time/chewing time), number of chews, chewing force and work were calculated for each extrudate products. Relative chewing force (highest EMG amplitude normalized to highest EMG amplitude for chewing gum) and relative work (EMG activity time × relative chewing force) parameters were calculated with relation to the chewing process of chewing gum. It is worth noting that the force and work computed this way are descriptive mastication parameters and not force and work in strict physical sense. Although the force generated by four muscle is linearly[44] relative to the measured voltage, the calibration varies between different subjects. The linear relationship between force generated by muscles and measured voltage was not measured in this study but this is a known correlation, which was first demonstrated by Prum *et al.*[44] All the parameters were computed separately for all four muscles monitored and the mean values were used in data analysis.

2.6. Bolus sample analyses

2.6.1 Saliva uptake. Saliva uptake of the bolus was determined based on the moisture contents of extrudates and their bolus samples.[28] Wet bolus samples were collected from 26 individual participants during mastication trial. A 0.5 g bolus from each participants was weighed in an aluminum moisture cup without mixing with each other. Moisture cups containing bolus samples were then placed in an oven at 105 °C. After overnight drying, bolus was weighed again. Saliva uptake was determined as the difference between the moisture content of boluses and the moisture content of extrudates. The data first calculated (average of three replicates) for each individual participants, and then reported as mean ± SD of 26 participants for each extrudate products.

2.6.2. Particle size distribution. Disintegration of the extrudates into particles was examined for the bolus samples (n = 26) using the protocol published by Alam *et al.*[28] The bolus samples (from 26 individuals participants) were diluted in a beaker with 100 ml of water, mixed with constant magnetic stirring of 220 rpm for 25 min. Diluted solution is then left to stand for 5 min in order to allow larger particles to settle to the bottom. Turbid liquid containing the smallest particles that could not be imaged was removed. A 100 ml of water was added again to increase the sample volume. The liquids containing bolus particles were poured onto Petri dishes for imaging. Around 7 to 8 Petri dishes were needed depending on the sample. The position of the particles were adjusted by moving/dragging them (when needed, if touched each other) over Petri dishes so that they were as little as possible in contact with each other. Digital images were taken of each Petri dish. Particle areas were determined using Cell^P imaging software (Olympus, Germany). Particle area distribution was visualized by a set of granulometric curves.

2.6.3. Viscosity. The viscosity of the bolus sample was analysed according to the method described for ground extrudates in section (2.4.4. Viscosity). Bolus samples were collected from a single chewer, who was not one of the 26 mastication trial participants. About 8 g of bolus (obtained from same person) sample was added to 25 ml of distilled water to reach the desired concentration (14% moisture basis). The adjusted weight of the sample (8.38–8.89 g) and water (21.11–21.62 g) was varied due to their moisture contents (48.66–50.42%). The data reported as mean ± SD values of three replicates.

2.7. Statistical analyses

Overall differences between study products were assessed using one-way ANOVA, and HSD Tukey was used as a *post hoc* analysis for pairwise comparison. Multivariate analysis of variance (MANOVA) was performed in order to see the joint and individual effect of each variables. Strongest effect indicated by Wilks Λ value close to zero, partial η^2 value close to unity and p less than 0.05. Linear correlations between different variables were calculated using 2-tailed Pearson bivariate correlation with significance level of 0.05. Statistical analyses were conducted using SPSS Statistics 24 (SPSS Inc., Chicago, USA) software.

3. Results

3.1. Chemical composition

The chemical compositions of the extrudates are shown in Table 3. The two extrudates with added rye bran (FBE and NBE) had almost identical compositions, and were clearly different from the EFE. EFE had 8% total DF, 6.5% protein and 78% starch. Both the extrudates with added rye bran had 22% total DF and 10% protein, whereas FBE had slightly less (54% *vs.* 57%) starch ($p < 0.05$). FBE had slightly lower insoluble (14% *vs.* 16%) DF ($p < 0.05$) and higher soluble DF (5.9% *vs.* 8.0%) compared to NBE. FBE had lower pH ($p < 0.05$) as compared to NBE and EFE (4.4 *vs.* 6.3 and 6.1, respectively). TTA varied between 1.5 (EFE) and 13 (FBE), whereas TTA of NBE was 2.5 ml.

3.1.1. Acidity, microbial and dextran analysis. Before fermentation, the measured pH of the bran was 6.3, while TTA was 2.0 (ml). The LAB counts in MRS agar was 6.4 log cfu g^{-1}, cell densities of yeasts and molds (YM agar) were less than 2 log cfu g^{-1}, cell densities of aerobic heterotrophic bacteria or fungi (PCA agar) were 3.0 log cfu g^{-1} and total spore forming aerobic bacteria count was less than 2 log cfu g^{-1}. After 20 h of fermentation at 25 °C, the pH and TTA of the bran was 4.1 and 9.7 (ml), respectively. The observed LAB count was 9.3 log cfu g^{-1}, yeasts and molds counts were less than 4 log cfu g^{-1}, aerobic heterotrophic bacteria or fungi were less than 3.0 log cfu g^{-1} and total spore forming aerobic bacteria count was 2 log cfu g^{-1}. The dextran content of fermented rye bran was 3% (3 g per 100 g bran; dry weight). Dextran was also formed in the control bran 0.8% (0.8 g per 100 g bran; dry weight) due to the free sucrose available in native rye bran.

3.2. Structural properties

FBE were notably more expanded than the NBE ($p < 0.05$), even though they had similar bran-flour ratios (Table 4). EFE were more expanded than both bran-added NBE and FBE ($p < 0.05$). The specific length of FBE was significantly higher than that of NBE ($p < 0.05$). Overall, bran addition resulted in reduced expansion and increased density, but this effect was less pronounced in FBE. MANOVA results showed that extrudates were significantly different due to the combined effect of expansion, specific length, piece density and DF content ($p < 0.05$), (Table 6a). There was a significant difference ($p < 0.05$) between samples for each individual variable with partial η^2 varying between 0.809 and 0.996 (data not shown in Table 6). DF content had the strongest effect on the product followed by piece density and expansion rate than specific length. Moreover, all structural properties were jointly and individually influenced ($p < 0.05$) by DF content (Table 6f). Expansion rate was negatively influenced by insoluble DF ($r = -0.83$, $p < 0.05$), indicating that the higher the insoluble DF in the extrudates, the lower the expansion was.

Table 3 Compositional analysis of EFE, NBE, FBE

	EFE	NBE	FBE
Moisture (%)	8.9 ± 0.0 ^{c}	8.0 ± 0.0 ^{b}	7.0 ± 0.1 ^{a}
Protein (%)	6.5 ± 0.1 ^{a}	10 ± 0.0 ^{b}	10 ± 0.1 ^{b}
Starch (%)	78 ± 0.2 ^{c}	57 ± 0.0 ^{b}	54 ± 0.3 ^{a}
Total dietary fiber (%)	8.0 ± 0.3 ^{a}	22 ± 0.9 ^{b}	22 ± 0.0 ^{b}
Insoluble dietary fiber (%)	4.5 ± 0.1 ^{a}	16 ± 0.0 ^{c}	14 ± 0.2 ^{b}
Soluble dietary fiber (%)	3.5 ± 0.2 ^{a}	5.9 ± 0.9 ^{b}	8.0 ± 0.2 ^{b}
Fat (%)	0.61 ± 0.00 ^{a}	1.5 ± 0.0 ^{c}	1.4 ± 0.0 ^{b}
pH	6.1 ± 0.1 ^{b}	6.3 ± 0.2 ^{b}	4.4 ± 0.0 ^{a}
TTA (ml)	1.5 ± 0.0 ^{a}	2.5 ± 0.0 ^{b}	13 ± 0.0 ^{c}

Based on dry weight; TTA: titratable acidity by 0.1 M NaOH. Values followed by different letters (a–c) in the same row were significantly different ($p < 0.05$).

Table 4 Structural and textural properties of EFE, NBE and FBE

	EFE	NBE	FBE
Macrostructure			
Expansion rate (%)	525 ± 32 ^{c}	369 ± 17 ^{a}	414 ± 18 ^{b}
Specific length (m kg^{-1})	48 ± 3.9 ^{a}	49 ± 2.4 ^{a}	63 ± 3.9 ^{b}
Piece density (kg m^{-3})	109 ± 13 ^{a}	213 ± 19 ^{c}	133 ± 11 ^{b}
Texture			
Hardness (N)	36 ± 4.7 ^{b}	49 ± 5.5 ^{c}	19 ± 2.3 ^{a}
Number of peaks	96 ± 3 ^{b}	85 ± 3 ^{a}	125 ± 3 ^{c}
Crispiness work (Nmm)	1.6 ± 0.3 ^{b}	2.2 ± 0.3 ^{c}	0.48 ± 0.07 ^{a}
Crispiness index ($\times 10^{-3}$)	4.7 ± 1.2 ^{b}	2.3 ± 0.4 ^{a}	43 ± 10.1 ^{c}

Values followed by different letters (a–c) in the same row were significantly different ($p < 0.05$).

3.3. Textural and sensory properties

NBE had the highest instrumental hardness (49 N), which was dramatically reduced by bran fermentation, even to a lower level than EFE (19 N *vs.* 36 N) (Table 4). As expected, rye bran addition resulted in lower crispiness (C_i = 0.002 *vs.* 0.005, C_w = 2.2 Nmm *vs.* 1.6 Nmm) in NBE compared to EFE. However, EPS-fermentation significantly increased the crispiness (C_i = 0.043 and C_w = 0.48 Nmm) of FBE compared to EFE and NBE ($p < 0.05$). Extrudates were significantly different ($p < 0.05$) due to the combined (Table 6b) effect of hardness, crispiness work, crispiness index and DF content. There was a significant difference ($p < 0.05$) between extrudates for each individual variable, with partial η^2 varying between 0.973 and 0.996 (data not shown in Table 6). Furthermore, all textural properties were jointly (Table 6g) and individually influenced by DF content ($p < 0.05$). EFE and FBE did not differ in perceived hardness and crispiness, whereas NBE was perceived to be the hardest and least crispy sample ($p < 0.05$), (Fig. 3). FBE was perceived as less coarse but had similar sliminess when compared to NBE. In MANOVA analysis, a combined effect of all sensory properties and DF content was observed (Table 6c). The samples were differed regarding all the variables except sliminess and thickness ($p < 0.05$) with partial η^2 varying between 0.238 and 0.476 (data not shown in Table 6). Intensity

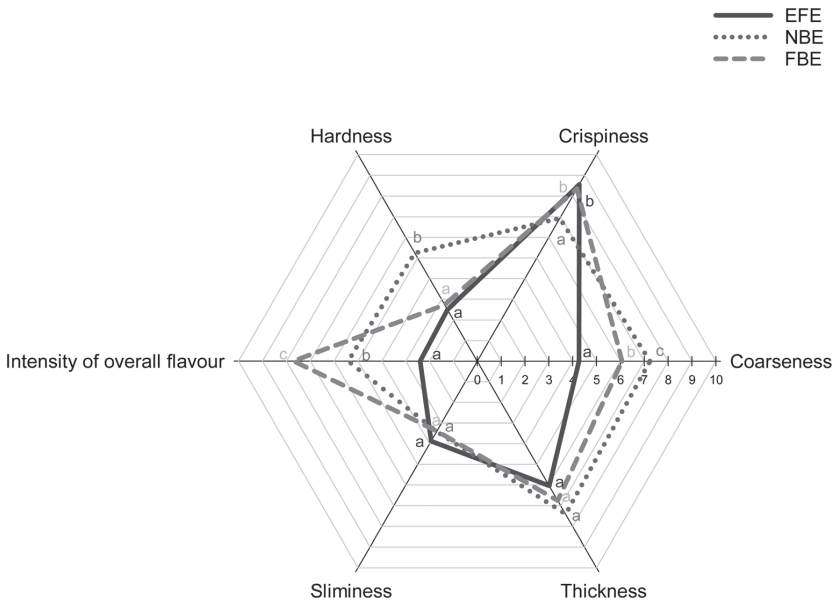

Fig. 3 Sensory attributes of EFE, NBE and FBE evaluated by the sensory panel. The different letters indicate statistically significant differences between products ($p < 0.05$).

of the overall flavour and DF content had the most significant effect (partial η^2 of 0.705 and 0.996, respectively).

3.4. Mastication properties

All extrudates had similar mastication profiles (Table 5). None of the mastication properties were significantly different ($p < 0.05$). The number of chews and chewing time were similar for all the studied products. Mastication properties and DF content had combined influence on the samples (Table 6d) but none of the mastication properties and DF content had individual effect ($p > 0.05$). Participants of mastication trial had significant effect on the mastication properties (Table 6m) indicating the existence of inter-individual variation. All the mastication parameters were significantly different based on the participants with partial η^2 varying between 0.843–0.929 (data not shown in Table 6).

Table 5 Mastication properties of EFE, NBE and FBE

	EFE	NBE	FBE
EMG activity time (s)	3.9 ± 1.5	4.3 ± 1.9	3.7 ± 1.7
Chewing time (s)	7.8 ± 3.4	8.4 ± 4.1	7.2 ± 3.7
Number of chews	11 ± 3.9	13 ± 5.9	11 ± 4.7
Duty cycle[a] (%)	52 ± 5.2	53 ± 5.0	53 ± 5.4
Relative total work[b] (%)	30 ± 13.1	34 ± 14.7	26 ± 12.6
Relative force/chew[b] (%)	76 ± 21.2	79 ± 24.6	71 ± 23.8
Saliva uptake (g per 100 g)	43 ± 7.4	43 ± 6.9	47 ± 8.8

The mean differences are NOT significant at the 0.05 level. [a] EMG activity time/chewing time. [b] Normalized to the corresponding values of a reference product (chewing gum).

3.5. Viscosity of the ground extrudates and boluses

When ground extrudate dispersions were compared, FBE were significantly less viscous ($p < 0.05$) than EFE and NBE, and EFE was the most viscous (Fig. 4). In the case of bolus samples, FBE was less viscous than EFE or NBE, but the difference was not statistically significant between EFE and NBE (Fig. 4). A pH drop was seen for EFE (6.0 *vs.* 4.3) and NBE (6.3 *vs.* 5.1) bolus samples when compared to dispersions of ground extruded products. However, the pH of both ground extruded products and bolus samples of FBE was below 5 (Fig. 4). In bolus samples, it took 2 hours to reach a constant viscosity level, after which all the clumps had been broken down and fully dissociated into the slurry.

3.6. Bolus particle size

There were more small particles in the bran-containing FBE and NBE boluses compared to the EFE bolus (Fig. 5). Crispy and low viscous FBE extrudates disintegrated rather easily during mastication, resulting in a larger number of small particles (Fig. 5d). The largest particle in the FBE bolus was 142 mm^2, whereas EFE and NBE had comparatively bigger particles, 218 mm^2 and 283 mm^2, respectively (Fig. 5d). The proportion of smaller particles (<10 mm^2) in FBE bolus was higher than in the EFE and NBE bolus. FBE had the highest share of small particles (≈80%) compared to EFE (≈57%) and NBE (≈66%). Mean particle area of all boluses varied between

Table 6 Multivariate analysis of variance results of the studied properties

	Wilk's Λ	Degree of freedom (hypothesis df, error df)	*p*	Partial η^2
Effect on products by:				
a. Macrostructure × DF	0.000412	$F(8, 144) = 869$	0.000	0.980
b. Texture × DF	0.000034	$F(8, 144) = 3075$	0.000	0.994
c. Sensory properties × DF	0.001367	$F(14, 54) = 100$	0.000	0.963
d. Mastication properties × DF	0.002682	$F(14, 138) = 181$	0.000	0.948
e. Bolus properties × DF	0.003342	$F(8, 144) = 293$	0.000	0.942
Effect of DF on:				
f. Macrostructure	0.011049	$F(15, 194) = 53$	0.000	0.777
g. Texture	0.001103	$F(15, 194) = 139$	0.000	0.897
h. Sensory properties	0.069424	$F(30, 102) = 3.2$	0.000	0.413
i. Mastication properties	0.563836	$F(30, 270) = 1.4$	0.093	0.108
j. Bolus properties	0.615383	$F(15, 194) = 2.5$	0.002	0.149
Effect on HI by:				
l. Macrostructure	0.008603	$F(33, 189) = 23.1$	0.000	0.795
k. Texture	0.000534	$F(33, 189) = 68.3$	0.000	0.919
Effect of participants on:				
o. Sensory properties	0.001332	$F(66, 107) = 3.9$	0.000	0.668
m. Mastication properties	0.000028	$F(150, 283) = 9.4$	0.000	0.825
n. Bolus properties	0.067112	$F(75, 150) = 2.9$	0.000	0.594

Strongest effect indicated by Wilks Λ value close to zero and partial η^2 value close to unity, $p < 0.05$.

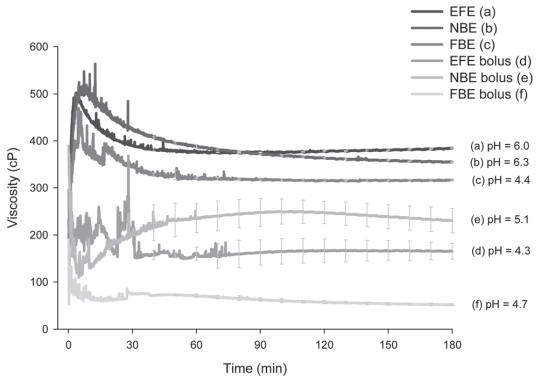

Fig. 4 Viscosity of ground (a, b, and c) and bolus (d, e, and f) samples of EFE, NBE and FBE.

0.027 and 0.034 mm^2. The extruded products did not differ in saliva uptake. Extrudates were significantly different due to the combined effect of bolus particle area, number of particles in the bolus, saliva uptake and fibre content ($p < 0.05$), (Table 6e). Among all the bolus properties, particle area had the most significant effect. Furthermore, all but saliva uptake were jointly (Table 6j) and individually influenced by fibre content ($p < 0.05$) with weak Wilks Λ and low partial η^2 values. However, all the bolus properties were significantly different based on the participants with partial η^2 varying from 0.502–0.678 (data not shown in Table 6), where saliva uptake had the strongest effect. There was a strong positive correlation between total particle area of each product and insoluble DF ($r = 0.99$, $p < 0.05$), suggesting that bran addition increased the number of particles in the boluses.

3.7. *In vitro* starch digestibility of the extrudates

The extent of starch hydrolysis in FBE was higher than in EFE and NBE ($p < 0.05$) (Fig. 6). Although containing 40% bran, NBE had a similar hydrolysis index to that of EFE. It was noticeable that the use of fermented rye bran in FBE resulted in higher starch digestion in the early phase. After 30 minutes of enzymatic incubation, FBE had a higher percentage of hydrolysed starch (80%) compared to EFE (70%) and NBE (73%). Macrostructure and texture both had significant effect on HI either jointly (Table 6l and k) or by individually with partial η^2 varying from 0.837–0.926 and 0.977–0.991 for macrostructure and texture, respectively (data not shown in Table 6). Interestingly, increase in DF content increased the HI ($r = 0.56$, $p < 0.05$), while increase in starch content reduced the HI ($r = -0.71$, $p < 0.05$).

4. Discussion

In the current study the use of fermented rye bran in rye extrudates resulted in a less hard and crispier texture, compared to native bran-containing extrudates. The positive effect of fermented rye bran on the textural properties of rye extrudates was also reported by Nikinmaa *et al.*,[22] who explained that when rye bran was fermented with dextran producing *Weissella confusa*, the bran surface was covered by a dextran layer. This might have reduced the premature air bubble ruptures by smoothing the surface of bran particles. We have shown in our previous paper Nikinmaa *et al.*,[22] that fermentation with *Weissella confusa* increased soluble pentosans from 1.5% to 2.8%. Solubilisation of DF from corn[45] and wheat bran[46] increased expansion and reduced density, respectively, whereas production of dextrans influenced product texture due to their ability to influence viscosity.[31] *In situ* dextran production (11–16 g kg^{-1}) in wheat sourdough increased the viscosity of the dough and improved the volume (up to 10%) and crumb softness (25–40%) of the corresponding bread. Therefore, both dextran production and solubilisation of DF might explain the improved structure observed in FBE.

Two high-DF (22%) rye extrudates had similar mastication properties to those of the extrudate (EFE) with less (8%) DF. Mastication properties of the three extrudates varying in structure and composition were not significantly different, although subtle differences were observed in the mastication effort measured as relative total work and relative force/chew. Although the product category was the same (*i.e.* all were dry puffed rye products), it was expected that the dense structure and hard texture of NBE would cause higher force/chew and longer chewing time. Probably the high inter-individual vari-

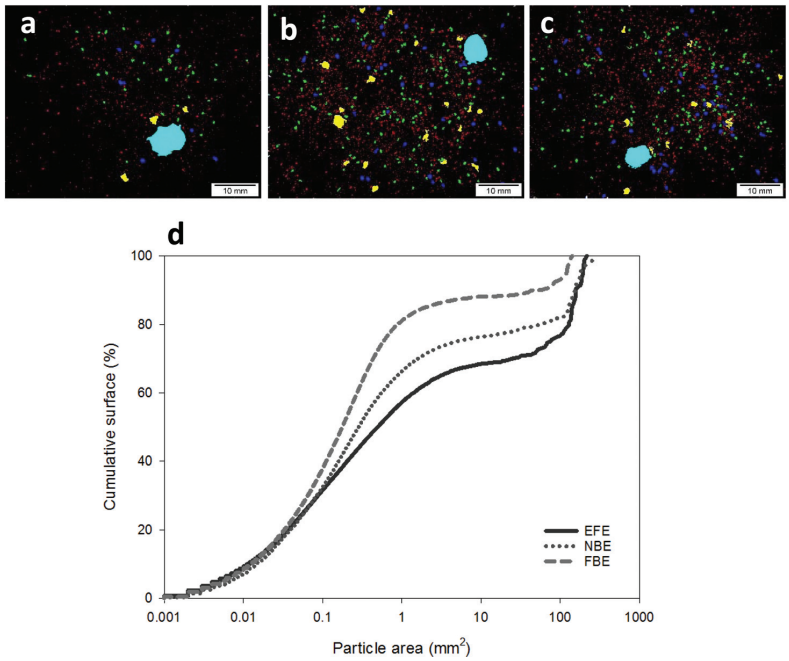

Fig. 5 Representative images of the masticated samples of (a) EFE (b) NBE and (c) FBE (the white bar is 10 mm). Particle area distributions of the samples are shown in (d), where the curves represent a cumulative percentage of the total area occupied by particles obtained from the boluses of 26 individual participants [color indication: red = 0.001–0.2 mm^2, green = 0.2–0.5 mm^2, blue = 0.5–1.0 mm^2, yellow = 1.0–10.0 mm^2, and cyan blue = 10–1000 mm^2].

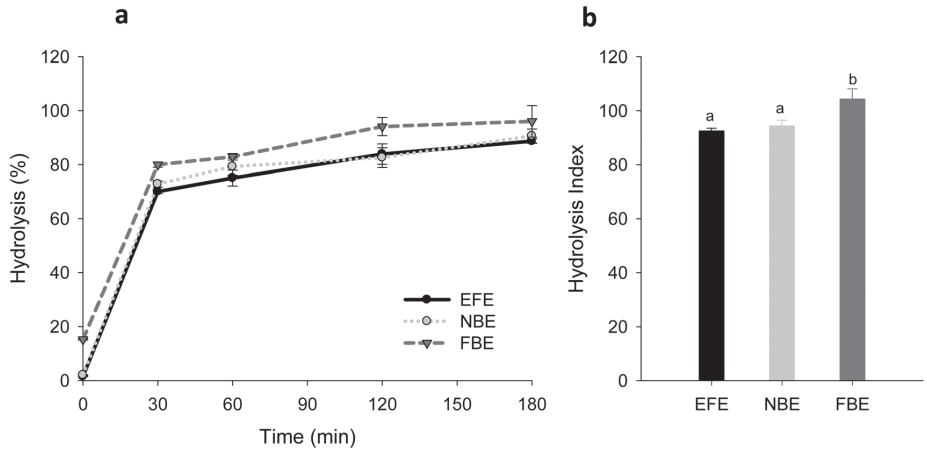

Fig. 6 (a) *In vitro* starch hydrolysis (% of hydrolysed starch) of EFE, NBE and FBE during 180 min incubation. (b) Hydrolysis indices (HI = (A_{uc} of extrudates/Average A_{uc} of white wheat bread) × 100) of extrudates. Values are means of three replicates ± SD. The bars marked with different letters were significantly different ($p < 0.05$).

ation overrode the possible small differences between products. We have previously reported that hard and dense extruded products required more mastication effort. However, in that study,[28] the effect of structure and texture on mastication was not observed within the same product type (puffs *vs.* rye bran added puffs). Significant differences in the mastication properties were only seen between different product types (puffed *vs.* flaked products). Proper insalivation and lubrica-

tion of the food is required in order to produce a swallowable bolus. It is assumed that hard and less crispy food products require longer mastication, which allows the food to be sufficiently lubricated with the saliva. However, in the current study, clear differences in hardness between the samples was not reflected in the mastication time or the number of chewing cycles.

Viscosity of the ground samples was 16–62% higher compared to bolus samples, demonstrating that the presence of salivary alpha amylase in bolus samples caused the main reduction. The hydrolysis of starch by salivary amylase was the main reason for this viscosity reduction. In this study, the low pH probably reduced the swelling capacity of ground FBE (pH = 4.4) compared to ground EFE (pH = 6.0) and NBE (pH = 6.3), which resulted in reduced viscosity. This is in agreement with the results of Okafor *et al.*[47] who observed that the swelling capacity of defatted black bean flour decreased when pH decreased from 6 to 4. Furthermore, FBE samples were crispy and mechanically weaker than EFE and NBE. It has been shown in our previous study that the crispy extrudates had low water solubility index (the amount of soluble components released from starch) compared to less crispy extrudates.[6]

Disintegration of the food during chewing, hydration, swelling and solubilization of different components, affects the viscosity of the bolus. In this study, FBE bolus was less viscous than the EFE and NBE boluses. The EFE and NBE boluses were more compact (not loose) than FBE boluses, indicating that more cohesive (visually observed) starch and protein phase was formed during mastication, which is in line with the study of Johansson *et al.*[48] and thus prevented swelling. In bolus samples, pH varied between 4.3–5.1. The activity of α-amylase reaches its peak at pH range 5.5–6.5 (Evans *et al.*).[49] Below this range enzyme activity starts to reduce and become close to zero at pH 4.5. Therefore, low viscosity of EFE bolus samples (pH = 4.3) than NBE bolus (pH = 5.1) could be explained by its low pH and in turn reduced swelling capacity. Moreover, EFE had the crispier structure than NBE, which probably disintegrated rather easily during constant stirring and due to the activity of α-amylase. For the same reason, crispiest FBE had the lowest viscosity. The mechanical energy of the gelatinised starch granules nearly lost when they come in contact with salivary α-amylase (Evans *et al.*).[49] The loss of mechanical energy make the granules weaker and accelerate disintegration. Amylases not only attack the surface of the granules but also the starch chains throughout the granule, which caused a drop in viscosity of the overall bolus due to a progressive weakening of granule structure.

Extrudates with added rye bran were disintegrated to smaller particles in the resulting bolus. The number of fine particles in the bolus was greater in NBE than in EFE, and a further increase of fine particles was seen in FBE. Sensory results also confirmed that the FBE bolus was perceived as less coarse than NBE. Rye bran addition made the extrudates less cohesive, probably due to incorporation of insoluble particles, which may have restricted the cluster formation during mastication. This further led to formation of smaller particles in the bolus. FBE bolus was paste like and loose, with the highest saliva uptake (47 g per 100 g of sample), whereas NBE as well as EFE boluses were compact and slightly dry (43 g per 100 g of both sample). Dissolution of polymers might have increased adhesion of saliva to bolus particles, resulting in loose bolus structure.[50] On the other hand, dryness in NBE and EFE was caused by the presence of comparatively intact starch-protein matrices which absorbed less saliva.

The hydrolysis index of the extrudates varied between 93 and 104, which is rather high compared to baked food such as bread. High HI is expectable after extrusion processing, as the starch present in the food matrix is totally degraded due to high shearing and temperature.[6,28,51,52] Increasing DF content from 8 to 22% using native bran in NBE (94) did not change HI compared to EFE (93), but using fermented bran did (104). Juntunen *et al.*[53] observed a decreasing trend in starch hydrolysis rate with an increased amount of DF in rye breads. The hydrolysis rate of starch was lower for high DF (29% DF) rye breads compared to traditional (15.2% DF) and endosperm rye bread (6.1% DF). By contrast, Rosén *et al.*[54] reported that although having less DF, the HI of endosperm rye bread (6.7% DF) was lower than that of whole grain rye (9.6% DF) and rye bran added (12.3% DF) bread. However, accessibility of starch to digestive enzymes may differ depending on processing methods and ingredients of food,[43] their structure and their particle size.[55] Structural differences within the products influenced the particle size of the ground extrudates as well as of the bolus samples. HI was positively influenced by crispiness index ($r = 0.98$, $p < 0.05$) and number of particles ($r = 0.85$, $p < 0.05$). We reported in our previous study[28] that the availability of starch for digestive enzymes in rye bran supplemented extrudates increased with the number of fine particles in the bolus. Insoluble bran particles probably disrupted the starch matrices in both NBE and FBE, but crispy texture in FBE led to an increase in smaller particles. Crispy extrudates were easily disintegrated, which enhanced the release of starch granules from the matrix, making them easily accessible to α-amylases. The presence of smaller particles in FBE increased the surface area, which led to increase susceptibility of starch granules to digestive enzymes, which is in agreement with the recent studies of Alam *et al.*[6] and Singh *et al.*[56] Therefore, texture, *i.e.* hardness and crispiness, had a prominent role in bolus formation and starch digestibility. We have previously reported that the use of coarse (D_{50} = 440 μm) rye bran decreased the HI compared to finely milled (D_{50} = 28 μm) rye bran.[6] However, in the current study coarse (D_{50} = 365 μm) rye bran was used in both NBE and FBE. Furthermore, comparatively low viscosity of FBE might also have influenced the HI by promoting diffusion in the digestive medium and consequently enhanced the susceptibility of enzymic breakdown of starch into sugars. A negative correlation was found between viscosity and HI ($r = -0.957$, $p < 0.05$), suggesting that the higher the viscosity, the lower the HI. Although EPS fermentation of the bran in the present study hindered the HI decreasing effect of coarse rye bran, it improved the palatability by means of providing better textural and structural properties. High DF content and good

textural properties of FBE make it a good alternative in the snack food category as a DF carrier.

5. Conclusion

The extrudates studied in this work were grouped into two pairs; the first (EFE and FBE) had similar macrostructure but distinct composition and the second pair (NBE and FBE) had distinct macrostructure but similar gross composition. The difference between EFE and FBE in instrumental crispiness was not reflected as a difference in perceived crispiness. This was probably due to the inter-subject variation, which prevented the detection of subtle textural differences. Possibly small differences in crispiness, detectable instrumentally, are not large enough to influence the perceived crispiness in general. Fermentation of bran induced compositional changes (*e.g.* production of dextran and solubilization of DF), which resulted in structural changes making the FBE extrudates more fragile. This further led to reduce bolus particle size and higher *in vitro* starch digestibility. Although, there was less starch in the fermented bran product compared to the unfermented product, the structure-texture interplay overruled this difference by resulting in a more fragile, easy to digest structure. A snack product with high dietary fibre content and palatable texture was achieved with fermented rye bran addition. Compared to NBE, FBE had less hard and more expanded and crispy texture that is considered beneficial in this product category. From nutritional point of view, FBE had a disadvantage of having higher *in vitro* starch digestibility compared to EFE and NBE. However, a portion (30 g) of FBE provides as much as 6.6 g of DF and only 16.2 g starch. Therefore, the glycemic load is reasonably low while the portion contributes significantly to the recommended daily DF intake. Considering the palatable texture and high DF content, FBE is a potential healthy alternative to the snack product category.

Abbreviations

C_i	Crispiness index
C_w	Crispiness work
DF	Dietary fibre
EF	Endosperm rye flour
EFE	Endosperm rye flour extrudates
EMG	Electromyography
EPS	Exopolysaccharide
FB	Fermented rye bran
FBE	Fermented rye bran extrudates
F_{max}	Hardness
HI	Starch hydrolysis index
NB	Native bran
NBE	Native rye bran extrudates
TTA	Titratable acidity

Conflicts of interest

There are no conflicts to declare.

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